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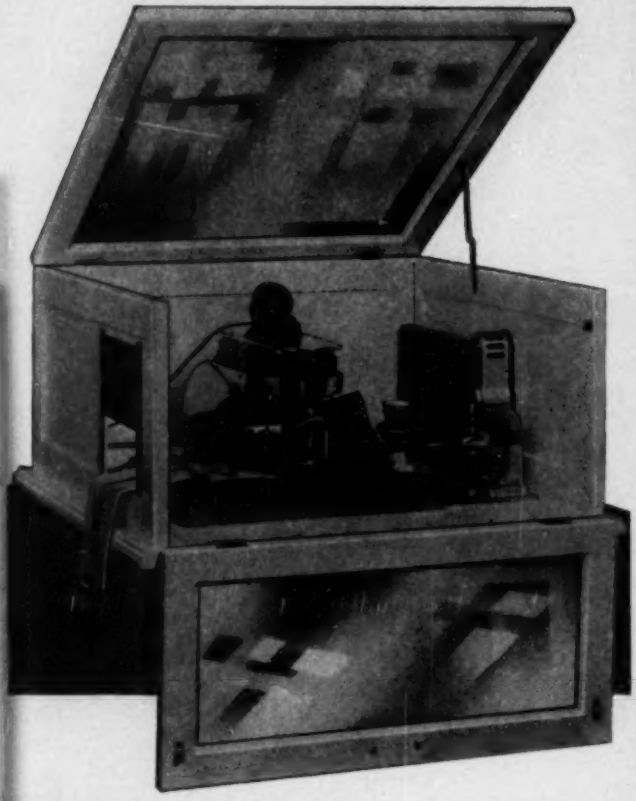

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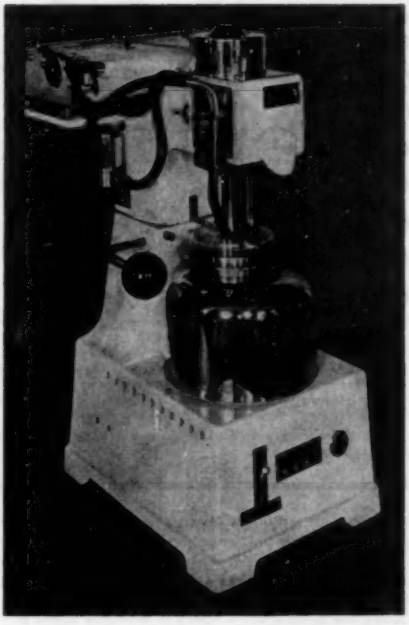

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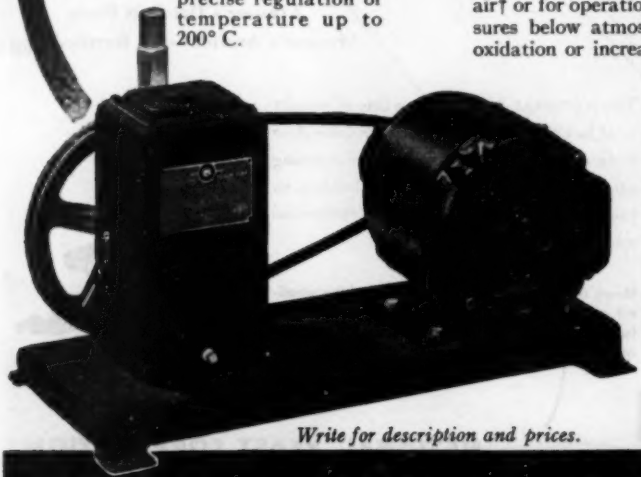

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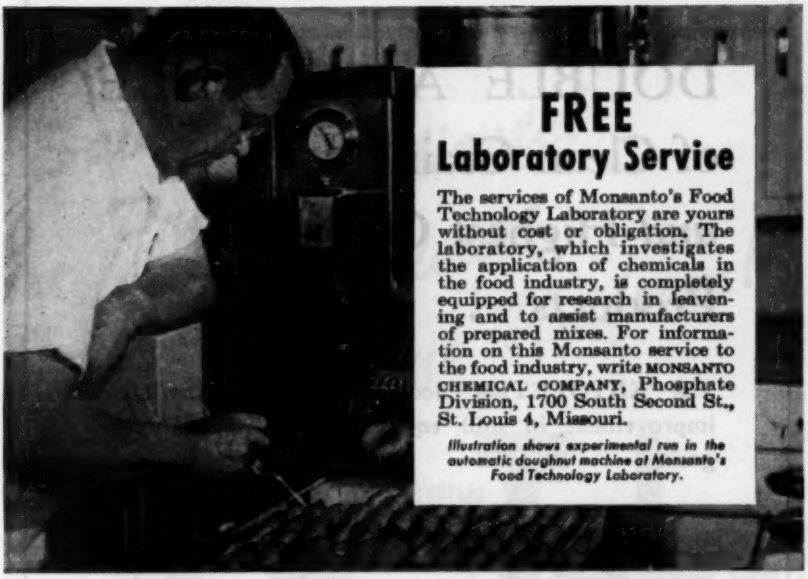

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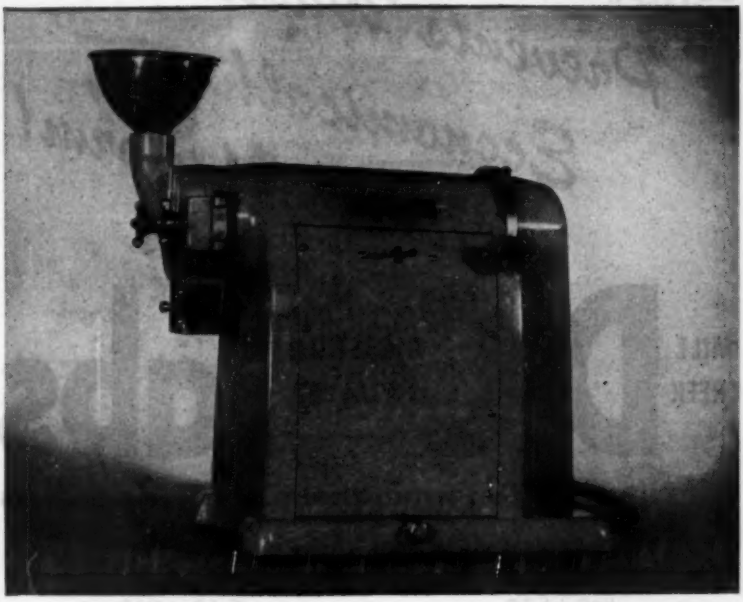

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CEREAL CHEMISTRY

VOL. XXVIII SEPTEMBER, 1951 No. 5

AN EVALUATION OF THE METHODS USED FOR DETERMINING THE BAKING QUALITY OF NONFAT DRY MILK SOLIDS [1,2]

BRUCE L. LARSON,[3] ROBERT JENNESS,[4] W. F. GEDDES,[4] and S. T. COULTER[5]

ABSTRACT

To determine the effects of variations in the manufacturing process upon the baking quality of nonfat milk solids and on the results of various methods which have been proposed for their evaluation, several samples of nonfat dry milk solids were prepared. Skim milk was preheated for 30 minutes at a series of temperatures ranging from 145° to 195°F., condensed to about 40% solids, and subsamples were spray-dried, superheated and spray-dried, and roller-dried.

All nonfat milk solids which were preheated at 145° and 155°F. were of poor baking quality even though those which were superheated and roller-dried received a high heat treatment.

Physical dough tests such as the farinograph and extensograph tests, and physical tests on reconstituted milks such as viscosity and solubility index, measure mainly the degree of casein destabilization which results in increased water absorption, an effect produced during the superheating and roller drying processes, which has little relationship to baking quality. The amount of undenatured serum protein, determined either by precipitation or by reducing group methods, was of most value as an index of the extent of the preheating treatment which the milk had received.

Milk solids are a desirable ingredient of bread since they supply valuable proteins, vitamins and minerals and frequently improve the appearance, as well as the palatability, toasting and keeping quality of the bread. The inclusion of milk in the bread formula, however, requires some modifications in the baking procedure. Moreover, several factors influence the baking quality of nonfat milk solids, the most important of which is adequate heat treatment of the skim milk before drying. Nonfat dry milk solids for use in breadmaking must have a high water absorbing capacity, yield doughs which do

[1] Manuscript received April 4, 1951. Presented at the Annual Meeting, May, 1951. Paper No. 2292 Scientific Journal Series, Minnesota Agricultural Experiment Station.

[2] The contents of this paper constitute a part of a thesis submitted by Bruce L. Larson to the Graduate Faculty of the University of Minnesota in partial fulfillment of the requirements for the Ph.D. degree.

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not soften or slacken materially during fermentation, and produce bread of satisfactory volume, crumb grain, and texture.

Since nonfat dry milk solids from different sources frequently show considerable variation in baking quality, bakers and dry milk manufacturers have been interested in the development of methods of evaluation. Experimental baking tests, physical dough tests, and physical and chemical tests on the milk have been used for this purpose. The factors influencing the production of high quality nonfat dry milk solids are not clearly understood, and the effect of the method of manufacture upon the various tests which have been used to determine dry milk quality has not been extensively investigated. The present investigation was undertaken to determine more fully the effects of these variables upon the results obtained by the tests used to evaluate dry milk. For this purpose, a series of samples has been prepared under controlled conditions, and these milks have been tested by various methods.

Historical

The beneficial effects of heat treatment on loaf volume and water-holding capacity, first demonstrated by Greenbank *et al.* (7), have been verified and extended by a number of workers (9, 16, 17, 26). The minimum heat treatment required for optimum loaf volume for most of this work seemed to be about 73°C. for 30 minutes. Recently, however, Ashworth and Krueger (3), using a formula that contained shortening, found that treatments of 80°C. for 15 or 30 minutes were necessary for optimum loaf volume. They also found that unheated skim milk produced larger loaves than did skim milk given heat treatments up to and including 77.5°C. for 15 minutes. Some workers (9, 16, 26) have observed that adequately heat-treated nonfat dry milk solids increased loaf volume above that of control loaves containing no milk. On the other hand, others (14, 17) have found depressions of loaf volume by any sample of nonfat dry milk solids. This apparent discrepancy doubtless arises from differences in baking formula, conditions and technique.

Unheated skim milk or nonfat dry milk solids prepared therefrom cause slackness and extreme extensibility of dough. This effect was first demonstrated by Skovholt and Bailey (27) with the farinograph. It appears that milk contains a factor which causes breakdown of the gluten matrix and that heat destroys or alters this factor. Thus, the problem has resolved itself into a search for such a factor and the elucidation of the effect of heat upon it. Casein apparently was eliminated by the finding of Stamberg and Bailey (29) that the dough-softening factor remained in milk serum after removal of the

casein with acid or rennet. These workers thought that the factor was a dialyzable constitutent of the serum, but Harland *et al.* (12) definitely showed it to be nondialyzable or, in other words, to be associated with the milk serum proteins (proteins other than casein).

Stamberg and Bailey (29) found by polarographic studies that raw skim milk gives typical crystine-cysteine polarographic curves, a property which it loses upon heat treatment. Consequently, they suggested that the dough-softening action of unheated milk is due to sulfhydryl groups which are altered or lost upon heat treatment. This suggestion is logical since it is well known that sulfhydryl compounds such as cysteine and glutathione cause slackening of dough. Farinograph curves for doughs containing cysteine or glutathione are similar to those for doughs containing unheated skim milk. Heat treatment produces an effect similar to removal of a reducing agent.

In baking tests and farinograph and extensograph studies with milk fractions Larsen *et al.* (17) confirmed the idea that the heat labile dough-softening and loaf volume-depressing agent resides in the milk serum proteins. Since these proteins are known to contain about 0.70% sulfur in the form of cysteine-cystine (18), it has been of interest to determine whether their deleterious effects on dough and bread and the beneficial effect of heat treatment involve their sulfhydryl groups.

The results obtained for sulfhydryl groups of the milk serum proteins depend on the method used for determining them. In unheated milk or milk serum protein sols the sulfhydryl groups are not sufficiently active or available to reduce nitroprusside (22) or thiamine disulfide (10). They do, however, reduce iodine; in fact, iodimetric titration of these groups in unheated β-lactoglobulin agrees closely with its known cysteine content (20). Heat treatment causes unfolding and reorientation of the protein in such a way that some of the sulfhydryl groups become available to such reagents as nitroprusside and thiamine disulfide. The total quantity as determined by *o*-iodosobenzoate and iodine does not decrease in the absence of oxygen but decreases markedly if oxygen is present during or following heat treatment (19, 20). In the case of milk serum protein heated in air, the decrease in total titratable sulfhydryl groups parallels the improvement in dough characteristics and loaf volume produced by heat treatment (18); however, it has never been established unequivocally that the sulfhydryl groups are directly responsible for the improvement noted.

Heat treatment of milk renders some of the serum proteins precipitable by acid at pH 4.6 (24) and by saturated sodium chloride (11). About 20% of these proteins is not rendered acid-precipitable by

any heat treatment (24). Sodium chloride precipitates about 20% of the serum proteins along with the casein from unheated milk and precipitates nearly all of them from adequately heated milk. The increase in precipitability has been used by Harland *et al.* (11) and Geddes *et al.* (6) as a measure of the extent of the denaturation of the serum proteins and of the improvement in baking quality.

Heat treatment causes agglomeration of the caseinate micelles in milk (23). This agglomeration with the consequent tendency to form a gel structure is important in relation to the water-holding capacity of dry milk in dough. The physico-chemical mechanism by which heating exerts this effect on the caseinate in not understood. Its progress can be followed by viscosity measurements. Greenbank *et al.* (7) and later Grewe and Holm (9) suggested a determination of the viscosity of 40% dispersions of milk powder in water as a criterion of baking quality. Their work led them to the belief that there is a close relationship between viscosity and baking quality of spray-dried powders that had been subjected to various preheating treatments. Skovholt and Bailey (26) and Johnson and Ward (16) found that the relation between baking quality and viscosity was not especially close.

Certain constituents of milk have detrimental effects on baking quality which are not eliminated by heat treatment. Thus the experiments of Larsen *et al.* (17) showed that casein, reconstituted as a calcium phosphocaseinate sol resembling that in milk, significantly depresses loaf volume and softens dough. Van Dam *et al.* (31) have also found that the loaf volume depression is associated with the calcium caseinate-phosphate. These workers contend that acid-precipitated casein is without effect on loaf volume, that calcium caseinate is an improver, and that calcium caseinate-phosphate centrifuged from milk with the Sharples supercentrifuge is a depressant. Van Dam *et al.* did not study the effect of heat treatment, but Larsen *et al.* (17) found that the baking quality of calcium phosphocaseinate sols was not improved by heat treatment. Van Dam *et al.* found also that the supercentrifuged whey was without effect on loaf volume, an observation which is difficult to reconcile with the findings of the various American workers that either acid or rennet whey depresses loaf volume.

Lactose also was found by Larsen *et al.* (17) to depress loaf volume although it did not soften dough. The effect of lactose cannot be overcome by heat treatment and the reason for its depressing effect is not understood.

Materials and Methods

Preparation of Nonfat Dry Milk Samples. On successive days in November, 170-lb. lots of pooled fresh raw skim milk were obtained from a commercial source. On arrival, the milk was warmed to 80°F. in a water bath to bring all the samples to a constant starting temperature. The milk was heated with stirring in two 10-gal. milk cans in a stream-heated water bath, brought to the desired temperature in 8 to 12 minutes, held for 30 minutes, and cooled to 140°F. in 5 to 8 minutes. Preheating temperatures of 145°, 155°, 165°, 175°, 185°, and 195°F. were used on successive mornings. The milk was condensed to about 40% solids in a Rogers' stainless steel vacuum pan and divided into three equal parts. One part was immediately spray-dried in a laboratory model spray drier. The second fraction was given a superheating treatment by heating the condensed milk with constant stirring in a steam-heated water bath to 170–175°F. The milk was held at this temperature until the viscosity was approximately doubled; it was then cooled and spray-dried. The third portion was diluted with three parts of distilled water, dried on a Buflovak laboratory model roller drier, and the sheets of dried milk pulverized in a hammer mill. The dry powders were sealed in No. 2 cans and stored in a cold room at 0°F. until analyses could be made. Because of difficulties with the roller drier on the first day, a 145°F. roller powder was not obtained; thus, a total of 17 samples were prepared.

In addition 24 samples of commercial nonfat dry milk solids of unknown history and variable baking quality were obtained from the American Dry Milk Institute. These samples were tested only by the *o*-iodosobenzoate-iodine method of Larson and Jenness (19, 20).

Experimental Baking Test. A standardized laboratory baking test employing 6 g. of milk solids per 100 g. of flour was conducted according to the method of Larsen *et al.* (17) to evaluate the quality of the dry milk samples. Duplicate loaves of bread were baked using a hard red spring wheat flour at three different bromate levels (1, 2, and 3 mg. per 100 g. of flour). A double portion of the dough formula was mixed in a Swanson-type mixer for 2.5 minutes, two 150 g. portions of dough scaled off for a 2 and 3 hour fermentation period, respectively, both portions proofed for 55 minutes and baked for 25 minutes at 230°C. The doughs were all brought to an absorption level necessary to give the desired consistency when removed from the mixer. The volume of the loaves was determined one hour after baking and they were scored for type, crust color, and crumb grain, texture and color the next day.

Physical Dough Tests. Two farinograph procedures and the Brabender Extensograph were employed, using the same lot of hard red spring wheat flour as was selected for the experimental baking test.

Farinograph tests were conducted by the method of Larsen *et al.* (17) in which the consistency of 480 g. of unleavened salted doughs (containing 12% of nonfat milk solids and 2% salt, both on a flour basis) at a constant absorption is measured at the point of maximum dough development and also after mixing at 30°C. for 25 minutes. The constant absorption employed is that required to yield a dough prepared with nonfat dry milk solids of high quality which will have a consistency of 500 Brabender units at maximum development.

The farinograph method of Hoffman *et al.* (15) which utilizes a mixture of equal parts of flour and nonfat dry milk solids was also used on these samples. Even though the method of Hoffman *et al.* was designed originally for roller powders employing an initial absorption of 65% for the milk powder, the same procedure was also used for the spray and superheated spray powders at a lower initial absorption, 30 and 40%, respectively. The absorption of the milk powder as well as the development time (minutes to reach the 500 line) was recorded for each sample.

The extensograph method of Larsen *et al.* (17) which measures the extensibility and resistance to extension of a dough containing milk was also employed. In this method the dough is prepared in the farinograph in exactly the same manner as for the farinograph method of Larsen *et al.* (17) except that each milk powder is prepared in a 480 g. dough with a consistency of 500 Brabender units. The dough is scaled into two 150 g. portions, each of which is rounded, molded, incubated and extended at rest intervals of 45, 90, and 135 minutes at 30°C. The extensibility and resistance to extension of the dough at each time is noted from the extensograph curves, and the two results averaged for each time.

Physical Tests on Reconstituted Milk. The solubility index of the dry milk powders was determined according to the method of the American Dry Milk Institute (1).

The relative viscosity of 7.5 ml. of a 10% dispersion (10 g. milk solids per 100 ml. solution) reconstituted at 25°C. was determined by means of an Ostwald pipette having a flow time for distilled water of 150 seconds. The milk sample was filtered through a fairly loose cotton plug before determining the viscosity.

The absolute viscosity was determined on a 20% suspension (20 g. of milk solids per 80 ml. of water) of the milk solids at 25°C. by means of the Brookfield rotating spindle viscosimeter (model LVF). Since

the 20% reconstituted samples exhibited a high degree of thixotropy, it was necessary to compare the viscosities at a high rotational speed (60 r.p.m.) of the spindle. Both viscosity tests were made 2 hours after the start of the reconstitution process.

Chemical Milk Tests. The amount of undenatured serum protein in a 10% (10 g. milk solids per 100 ml. solution) reconstituted sample was determined both by the Harland and Ashworth (11) procedure, which determines the serum proteins not precipitated by saturated sodium chloride, and by the Rowland (24) procedure which determines the serum proteins not precipitated when casein is removed from the system by acidification. For the Rowland method, the serum protein fraction was taken to be that non-precipitable by acetate buffer at pH 4.6 but precipitated by 12% trichloracetic acid.

Reducing groups were determined by the *o*-iodosobenzoate-iodine procedure of Larson and Jenness (19, 20), the thiamine disulfide method of Harland and Ashworth (10) and the ferricyanide method of Crowe *et al.* (4). The non-ascorbic reducing power of the milk determined by the *o*-iodosobenzoate-iodine procedure was evaluated by titrations 2 and 24 hours after reconstitution of a 10% solution (10 g. milk solids per 100 ml. solution), the milk being stored in an icebox at 4°C. during the interim.

Results

Experimental Baking Texts. The results of the baking tests are recorded in Table I. In general, the best results were obtained at the bromate level of 2 mg. with a 2 hour fermentation period. The loaf volumes and quality scores are higher for the 3 mg. bromate level; however, there was a decided tendency for these loaves to show internal holes, thereby increasing the volume. The 2 mg. bromate level and 2 hour fermentation period also exhibited the best differentiation between the milk powders. Figure 1 shows the loaf volumes obtained with these optimal conditions plotted against the perheating temperature. The great influence of the preheating process on the loaf volume is apparent, and it is noteworthy that the drastic heat treatments given the superheated and roller powders did not overcome the lack of an adequate preheating temperature. Thus the 145° and 155°F. spray, superheated spray and roller powders were of poor baking quality even though the last two received high heat treatments before or during the drying process. The preheating process and method of manufacture did not seem to affect appreciably or consistently the internal quality characteristics or the type score of the loaves.

These samples also were baked into 1 lb. loaves by the American Dry Milk Institute at a level of 6% milk solids using a constant

TABLE I

EFFECT OF PREHEATING TEMPERATURE AND METHOD OF MANUFACTURE OF NONFAT DRY MILK SOLIDS ON THE BAKING QUALITY OF DOUGHS CONTAINING THEM AT A 6% LEVEL (FLOUR BASIS) [1]

Type of Powder	30 Min. Preheat Temp.	Fermentation Time	Absorption	Mg. Bromate per 100 g. Flour					
				1		2		3	
				Loaf Volume	Quality Score[2]	Loaf Volume	Quality Score[2]	Loaf Volume	Quality Score[2]
	°F.	hrs.	%	cc.		cc.		cc.	
Spray	145	2	65	712	88.2	728	89.2	735	91.0
	155	2	65	695	84.5	730	89.0	730	90.0
	165	2	66	785	96.5	810	99.0	838	101.8
	175	2	67	775	94.5	798	97.8	832	102.2
	185	2	67	735	91.5	798	99.2	845	101.5
	195	2	67	745	93.5	808	99.8	815	100.0
	145	3	65	678	83.8	668	84.2	662	81.8
	155	3	65	690	85.5	705	86.5	672	82.8
	165	3	66	738	94.2	752	95.2	708	82.6
	175	3	67	742	93.2	752	94.2	732	89.2
	185	3	67	725	89.5	738	92.2	722	91.8
	195	3	67	700	88.0	748	92.2	722	87.8
Superheat Spray	145	2	65	708	87.5	738	91.8	750	93.5
	155	2	65	722	85.8	745	87.5	780	96.0
	165	2	66	775	94.5	828	102.8	842	103.5
	175	2	67	760	93.0	825	94.5	842	100.8
	185	2	67	758	95.2	815	97.8	810	99.5
	195	2	67	748	92.8	820	100.5	838	102.8
	145	3	65	685	85.0	698	85.2	678	82.2
	155	3	65	698	86.8	710	87.5	698	86.8
	165	3	66	765	93.5	808	97.8	720	88.5
	175	3	67	730	91.5	758	95.8	738	91.8
	185	3	67	725	91.5	705	86.0	700	87.0
	195	3	67	748	94.2	722	89.8	702	87.8
Roller	155	2	65	715	88.5	755	92.0	748	91.8
	165	2	66	802	99.8	818	100.8	852	103.8
	175	2	67	810	97.5	822	99.8	890	105.0
	185	2	67	758	95.8	818	98.2	840	104.0
	195	2	67	775	96.0	838	101.8	838	104.2
	155	3	65	692	86.8	710	88.0	700	86.5
	165	3	66	765	98.0	775	95.0	720	87.5
	175	3	67	775	95.5	742	91.2	742	90.8
	185	3	67	770	97.5	745	91.2	738	91.2
	195	3	67	785	96.0	788	98.8	758	93.8
Control (No milk)		2	63	730	102.7	(0 mg. bromate)			
		3	63	751	97.6				

[1] Each figure represents the average of 2 duplicate loaves baked on different days.
[2] Quality Score = 0.1 (loaf volume − 200) + grain + texture + crumb color + loaf type + crust color. On the basis of a possible score of 10 for each of the 5 loaf characteristics.

absorption of 68%. The loaf volumes shown in Fig. 1 indicate that the two independent baking tests are in agreement.

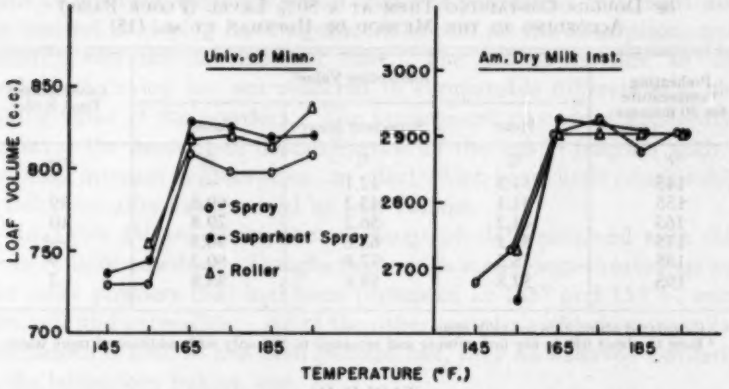

FIG. 1. Effect of the method of manufacture on loaf volume. The skim milk was heated for 30 minutes before drying at the indicated temperatures and the nonfat dry milk solids were incorporated in the baking formula at a 6% level (flour basis). Baking tests were conducted at the University of Minnesota and at the American Dry Milk Institute, Inc.

Physical Dough Tests. Farinograph data obtained by the method of Larsen *et al.* (17) and Hoffman *et al.* (15) are shown in Tables II and III, respectively. The method of Larsen *et al.* is useful to compare samples with different preheating temperatures prepared under

TABLE II

EFFECT OF PREHEATING TEMPERATURE AND METHOD OF MANUFACTURE OF NONFAT DRY MILK SOLIDS ON THE BEAVHIOR IN THE FARINOGRAPH OF DOUGHS CONTAINING THEM AT A 12% LEVEL (FLOUR BASIS) ACCORDING TO THE METHOD OF LARSEN ET AL. (17)

Preheating Temperature for 30 Minutes	Dough Consistency					
	Spray[1]		Superheat Spray[2]		Roller[3]	
	Maximum	25 Min. Reading	Maximum	25 Min. Reading	Maximum	25 Min. Reading
°F.	*Brabender Units*					
145	440	400	500	480	—	—
155	440	400	460	440	430	410
165	500	470	500	480	440	430
175	500	480	500	490	460	450
185	490	480	490	470	490	480
195	500	480	500	480	500	500

[1] 73% absorption.
[2] 74% absorption.
[3] 76% absorption.

TABLE III

EFFECT OF PREHEATING TEMPERATURE AND METHOD OF MANUFACTURE OF NONFAT DRY MILK SOLIDS ON THE BEHAVIOR IN THE FARINOGRAPH OF DOUGHS CONTAINING THEM AT A 50% LEVEL (FLOUR BASIS) ACCORDING TO THE METHOD OF HOFFMAN ET AL. (15)

Preheating Temperature for 30 minutes	Absorption Value[1]			Development Time Roller
	Spray	Superheat Spray	Roller	
°F.	%	%	%	min.
145	31.3	42.1	—	—
155	34.1	43.2	69.8	19
165	44.2	56.2	79.8	10
175	52.2	63.8	90.8	—[2]
185	55.5	57.8	80.2	9
195	52.8	59.8	83.8	7

[1] Calculated absorption for milk alone.
[2] Rose to about 650 in the first minute and returned to 500 only with addition of more water.

TABLE IV

EFFECT OF PREHEATING TEMPERATURE AND METHOD OF MANUFACTURE OF NONFAT DRY MILK SOLIDS ON THE BEHAVIOR IN THE EXTENSOGRAPH OF DOUGHS CONTAINING THEM AT A 12% LEVEL (FLOUR BASIS)

Preheating Temperature for 30 Minutes	Rest Period					
	45 Min.		90 Min.		135 Min.	
	E[1]	H[2]	E	H	E	H
Spray						
145[3]	—	—	—	—	—	—
155[3]	—	—	—	—	—	—
165	20	250	13	600	10	700
175	19	360	13	680	10	720
185	18	330	13	710	10	730
195	17	440	12	670	12	750
Superheat Spray						
145[3]	—	—	—	—	—	—
155	25	160	20	470	—	—
165	18	320	12	630	10	690
175	18	470	14	720	12	760
185	18	360	13	650	10	620
195	17	300	12	600	10	720
Roller						
155	22	270	15	620	14	700
165	18	340	12	680	10	770
175	19	410	12	710	10	810
185	19	300	11	640	10	710
195	18	350	12	690	11	730
Control (no milk solids)	22	490	16	600	19	600

[1] E is extenisibility in Brabender Units.
[2] H is resistance to extension in Brabender Units.
[3] These doughs were too soft to handle in the extensograph.

similar conditions; however, since it reflects differences in absorption it is of little use for evaluating a given sample of unknown history. Both farinograph methods reveal that the preheating treatment and the method of drying have significant effects on the absorption, consistency, and the development time. The differences due to the method of drying are not reflected in comparable differences in the baking value of the powders. The farinograph procedure apparently measures the amount of destabilization of the casein fraction with a resultant increase in absorption, an effect which bears little relationship to baking quality as measured by loaf volume.

In Table IV are shown the extensograph data obtained with this series of milk powders. Doughs made with spray, superheated spray, and roller powders that had been preheated at 145° and 155°F. were very soft and extensible. All of the other samples yielded very similar extensograms and, as has been pointed out, they all behaved similarly in the laboratory baking test.

TABLE V

EFFECT OF PREHEATING TEMPERATURE AND METHOD OF MANUFACTURE OF NONFAT DRY MILK SOLIDS ON THE SOLUBILITY INDEX AND VISCOSITY OF MILK RECONSTITUTED THEREFROM

Preheating Temperature for 30 Minutes	Spray			Superheat Spray			Roller		
	Viscosity		Solubility Index[3]	Viscosity		Solubility Index[3]	Viscosity		Solubility Index[3]
	Relative[1]	Absolute[2]		Relative[1]	Absolute[2]		Relative[1]	Absolute[2]	
°F.		cp.	cc.		cp.	cc.		cp.	cc.
145	1.70	5.5	0.10	2.11	25	2.75	—	—	—
155	1.66	5.3	0.10	1.93	7	0.75	—	475	13.0
165	1.78	5.7	0.10	2.77	40	4.00	—	450	14.0
175	1.85	6.0	0.20	2.90	—	7.50	—	650	12.0
185	1.89	7.1	0.10	2.09	12	0.15	—	410	12.0
195	1.93	7.2	0.15	2.44	28	2.65	—	900	12.0

[1] Viscosity relative to water determined with an Ostwald pipette on a 10% suspension.
[2] Viscosity of a 20% suspension recorded in centipoises using a Brookfield rotating spindle viscosimeter.
[3] Method of American Dry Milk Institute (1).

Physical Tests on Reconstituted Milk. The solubility index and the viscosity determinations, for which data are shown in Table V, make it possible to differentiate roughly the spray, superheated spray and roller powders. These physical milk tests reflect the degree of casein destabilization, a factor which apparently bears little relationship to baking quality.

Chemical Tests on Reconstituted Milk. Figure 2(A) and Table VI show the effect of the method of manufacture and preheating temperature on the precipitability of the serum proteins as measured by the

methods of Harland and Ashworth (11) and Rowland (24). Since saturated sodium chloride precipitates a protein fraction that acidification to pH 4.6 does not, the values obtained by the Harland and Ashworth method are lower by a constant amount than those obtained by the Rowland procedure. The values for the roller powders are

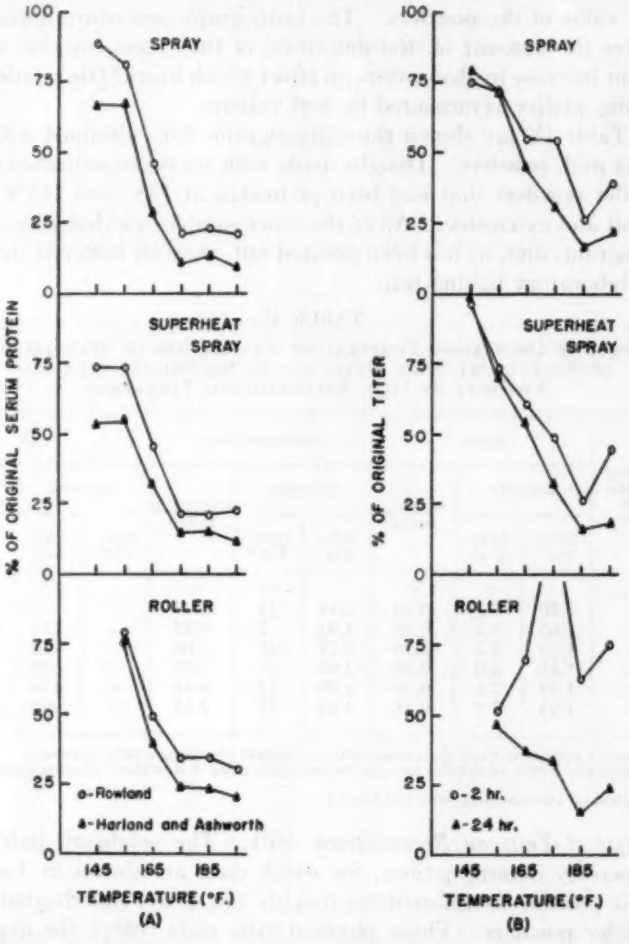

FIG. 2(A). Effect of the method of manufacture on the denaturation of the serum proteins. The amount of undenatured serum protein was determined in the reconstituted samples which had been preheated at the indicated temperatures for 30 minutes before drying. The percentage of undenatured serum protein, as determined by the Harland and Ashworth (11) and the Rowland (24) procedures, is calculated on the original amount determined by the Rowland method in the unheated skim milk.

FIG. 2(B). Effect of the method of manufacture on the non-ascorbic o-iodosobenzoate-iodine titration for protein reducing groups. The titer was determined at 2 and 24 hours after reconstitution for the samples which had been preheated at the indicated temperatures for 30 minutes before drying. The percentage of titer remaining is based on the titer of the original unheated skim milk.

TABLE VI

EFFECT OF PREHEATING TEMPERATURE AND METHOD OF MANUFACTURE OF NONFAT DRY MILK SOLIDS ON THE DENATURATION OF THE SERUM PROTEINS

Preheating Temperature for 30 Minutes	Original Skim Milk	Undenatured Serum Protein Nitrogen[1]					
		Spray		Superheat Spray		Roller	
		Rowland	Harland & Ashworth	Rowland	Harland & Ashworth	Rowland	Harland & Ashworth
°F.	mg./g.	mg./g.	mg./g.	mg./g.	mg./g.	mg./g.	mg./g.
145	10.3	9.2	6.8	7.7	5.5	—	—
155	9.3	7.6	6.7	6.9	5.1	7.3	7.1
165	9.5	4.5	2.8	4.3	3.0	4.7	3.8
175	10.5	2.2	1.2	2.4	1.5	3.6	2.7
185	8.5	2.0	1.1	1.8	1.2	3.0	2.0
195	9.8	2.0	0.8	2.3	1.1	3.0	2.0

[1] Results are expressed as mg. of undenatured serum protein nitrogen per g. of nonfat dry milk solids.

higher than for the spray powders probably due to the breakdown of the proteins into non-precipitable fragments during the high heating in the roller drying process. Harland and Ashworth developed their method for spray powders, but since the industry has been applying it to all types of powders, determinations were made on all of the samples. The results in Fig. 2(A) show that the preheating process is the important factor influencing the amount of non-denatured serum proteins found by both of the methods, and a close relationship is shown to the baking test.

In Table VII are given the data obtained on the series of milk samples by the various methods to determine reducing groups. The thiamine disulfide test does not appear to give an indication of anything pertinent other than to indicate a characteristic increase in titer in the highly heated milks. However, this test would be very dependent upon the holding history of the milk since Harland and Ashworth (10) have shown that heated milks on holding in the fluid state decrease in titer; thus a highly heated milk which was held in the fluid state before drying might have the same titer as a very low heated sample. Table VII and Fig. 2(B) show that the non-ascorbic *o*-iodosobenzoate-iodine, thiamine disulfide, and ferricyanide titers for roller powders are very high. In very highly heated milks protein-sugar interactions occur which cause more reducing matter to appear and this has an especially large effect on the ferricyanide titers (4). A characteristic decrease of the *o*-iodosobenzoate-iodine titer with increasing heat treatment was shown for all of the samples. Significant decreases in the *o*-iodosobenzoate-iodine titers occurred when the

samples that had been preheated at 165°F. or higher were held in the cold for 24 hours after reconstitution. Apparently oxidation of activated groups was arrested upon drying and continued upon reconstitution. The similarities in appearance of Figs. 2(A) and (B) show that there is a close relationship between the extent of serum protein denaturation as measured by the degree of precipitability by acid at pH 4.6 or saturated sodium chloride and by the *o*-iodosobenzoate-iodine titration method for protein reducing groups.

Inasmuch as the samples of poor baking quality had a high *o*-iodosobenzoate-iodine titer which did not decrease during holding for 24 hours, it was thought that the final titer and the decrease during the holding period might be a satisfactory index of the baking quality of dry milk solids. The test was applied to a series of 24 commercial samples of nonfat dry milk solids of unknown history and variable baking quality obtained from the American Dry Milk Institute. In Table VIII are shown the baking characteristics of these samples and the non-ascorbic *o*-iodosobenzoate-iodine titers determined at 1, 24, and 48 hours after reconstitution. It is apparent

TABLE VII

EFFECT OF PREHEATING TEMPERATURE AND METHOD OF MANUFACTURE OF NONFAT DRY MILK SOLIDS ON THEIR REDUCING GROUPS

Type of Powder	Preheating Temp. for 30 Minutes	Reducing Power			
		TDRS[1]	AFRS[2]	IBRS[3]	
				2 Hrs.	24 Hrs.
	°F.	*m.eq./100 g.*	*m.eq./100 g.*	*m.eq./100 g.*	*m.eq./100 g.*
Spray	145	0.008	0.120	0.211	0.223
	155	0.000	0.150	0.193	0.193
	165	0.060	0.120	0.156	0.130
	175	0.090	0.120	0.140	0.080
	185	0.069	0.130	0.076	0.057
	195	0.085	0.150	0.099	0.056
Superheat Spray	145	0.008	0.440	0.271	0.276
	155	0.000	0.150	0.193	0.187
	165	0.037	0.380	0.164	0.150
	175	0.067	0.290	0.125	0.082
	185	0.078	0.150	0.079	0.049
	195	0.062	0.280	0.116	0.048
Roller	155	0.060	—	0.137	0.127
	165	0.083	0.440	0.186	0.103
	175	0.062	0.480	0.305	0.088
	185	0.078	0.440	0.182	0.047
	195	0.088	0.350	0.190	0.060

[1] Thiamine disulfide reducing substances.
[2] Acid ferricyanide reducing substances.
[3] Substances that reduce o-iodosobenzoate-iodine (correction has been made for ascorbic acid).

TABLE VIII

BAKING PERFORMANCE AND *o*-IODOSOBENZOATE–IODINE REDUCING TITERS OF COMMERCIAL SAMPLES OF NONFAT DRY MILK SOLIDS OF UNKNOWN HISTORY

Sample No.	Type of Powder	*o*-Iodosobenzoate-Iodine Reducing Power[2]			Baking Performance
		1 Hr.	24 Hrs.	48 Hrs.	
		m.eq./100 g.	*m.eq./100 g.*	*m.eq./100 g.*	
1	Roller	0.243	0.153	0.134	Poor
2	Spray	0.238	0.151	0.150	Good
3	Spray	0.185	0.149	0.129	Good
4	Spray	0.132	0.129	0.129	Poor
5	Spray	0.165	0.121	0.111	Good
6	Spray	0.199	0.120	0.099	Good
7	Spray	0.226	0.185	0.174	Poor
8	Spray	0.200	0.120	0.094	Good
9	Roller	0.217	0.123	0.116	Good
10	Spray	0.236	0.194	0.177	Fair
11	Spray	0.203	0.152	0.121	Poor
12	Spray	0.144	0.101	0.084	Poor
13	Spray	0.164	0.086	0.093	Good
14	Spray	0.244	0.163	0.133	Good
15	Roller	0.235	0.121	0.106	Poor
16	Roller	0.221	0.095	0.030	Good
17	Roller	0.172	0.079	0.067	Fair
18	Roller	0.095	0.064	0.054	Good
19	Spray	0.169	0.089	0.063	Good
20	Spray	0.373	0.241	0.248	Poor
21	Spray	0.280	0.246	0.250	Poor
22	Spray	0.263	0.176	0.150	Fair
23	Roller	0.289	0.143	0.107	Good
24	Roller	0.302	0.156	0.134	Poor

[1] These samples were collected and their baking performance determined by the American Dry Milk Institute, Inc.

[2] Titrations made on a 10% solution (10 g. solids per 100 ml. solution) at 1, 24 and 48 hours after reconstitution. Correction has been made for ascorbic acid.

that all of them exhibited marked decreases in reducing titer with the exception of No. 4 which was of poor baking quality. However, many of the samples which showed a large decrease were also of poor baking quality. Those samples which had a very large reducing titer at all three of the time intervals were characteristically of poor baking quality; however, no definite classification of the milks into poor and good categories can be made since there are several notable exceptions. Thus the test does not seem to be applicable to the absolute evaluation of the baking quality of a sample nonfat dry milk solids of unknown history. The fact has been demonstrated, however, that reconstituted commercial nonfat dry milk solids exhibit a decrease in non-ascorbic reducing groups upon standing and that these reducing groups are probably sulfhydryl. No characteristic relationship was noted between the ascorbic acid content of the milks and the decrease in the non-ascorbic *o*-iodosobenzoate-iodine titer.

Discussion

The comparative studies with this series of nonfat dry milk solids show that results of each of the tests commonly used to evaluate baking quality are affected by the treatment of the milk during manufacturing. The preheating treatment given the fluid milk is of paramount importance in determining the baking quality. Milk samples preheated at 145° or 155°F. for 30 minutes were not of good baking quality even though they received high heat treatment during the subsequent superheating or roller drying processes. The relation of preheating temperature to baking quality found with this series is very similar to that previously reported from this laboratory (17). The minimum heat treatment for optimum loaf volume is between 155° and 165°F. for 30 minutes.

The heat treatment of the concentrated product in the superheating and roller processes has little effect on the denaturation of the serum proteins or on loaf characteristics, but considerably destabilizes the caseinate with a resulting increase in the water-holding capacity. Thus tests such as viscosity, solubility index, and to some extent the farinograph which measure the instability of the casein do not give a reliable indication of baking quality as measured by loaf volume, but they do reflect the increased absorption resulting from heat treatment. Of the physical dough tests, the extensograph method appears to offer the most promise of furnishing an indication of baking quality. In this test the dough is first mixed to a constant consistency in the farinograph therby eliminating variations due to differences in absorption. However, on the basis of this series of milks it is difficult to establish a significant level of extensibility and resistance to extension for a good baking quality dry milk sample. Also flours differ in their action, and before the test could be put on any kind of quantitative basis a "standard" flour as well as a standardization of extensographs would be needed. More information is needed on milks prepared by different methods of manufacture before the usefulness of the extensograph method can be determined. In addition, the extensograph is a very expensive instrument and the extensograph test is more time consuming than an actual baking test.

Methods measuring the extent of denaturation of the serum proteins, which parallels closely the baking quality, seem to offer considerable promise in evaluating dry milk for baking. The Rowland (24) and Harland and Ashworth (11) precipitation methods are equally applicable to this purpose. They have serious limitations, however, when used to evaluate a given sample of unknown history. In the first place, milks differ in total serum protein content (20) so that the absolute amount of undenatured serum protein in a sample unless it

is very low does not necessarily indicate the extent of heat treatment. Furthermore, it appears that roller drying may cause some protein degradation, the products of which appear as undenatured serum protein in the analysis.

Tests which determine the non-ascorbic reducing capacity of the reconstituted dry milk also have serious limitations. Thiamine disulfide measures only some of the more active sulfhydryl groups and since the amount of these depends not only on the extent of heat treatment but also on the opportunity given for oxidation, the method is not of much value in determining heat treatment alone. The ferricyanide method suffers from lack of specificity (4). Probably the best of these methods is the *o*-iodosobenzoate-iodine titration conducted 24 hours after reconstitution so that activated groups are oxidized. Only if opportunity is afforded for oxidation of the activated groups do the results give a valid indication of the extent of the heat treatment. The titration, like the precipitation methods, is limited in application to samples of unknown history because of differences in serum protein content among milks (20). It is further complicated by the fact that sulfhydryl content per unit of serum protein varies among milks (20).

It has never been shown conclusively that the actual content of undenatured serum protein determines the baking quality of the milk. In the present study no characteristic differences were found in the baking quality of the samples that had been preheated above 165°F. for 30 minutes even though they differed in the amount of undenatured serum proteins present. On the other hand, Ashworth and Krueger (2) have shown a poor correlation between loaf volume and the amount of undenatured serum protein as determined by the Harland and Ashworth (11) method when the heat treatment was insufficient to denature 50% of the serum proteins; however, a significant correlation was shown above this level. It is possible that the absolute amount of undenatured serum protein is not the actual factor responsible for baking performance, but rather that serum protein denaturation parallels some other change that accounts for the improvement in baking quality by heat treatment. Thus, the extent of the serum protein denaturation may be considered an index of the heat treatment. Such an index would be of the most value if expressed as percentage denaturation of the serum proteins originally present. In the determinations reported in this paper it was possible to calculate the results in this way because data were available for the total serum protein content as determined on the raw milk. With the methods available at present, however, it is not possible to express the results in this way for a sample of nonfat dry milk solids of unknown history, but only

to express them in terms of concentration of undenatured serum proteins remaining. Development of methods for determining the total serum protein content (both denatured and undenatured) of a dry milk sample would be desirable.

Variability in baking procedure may well be the most important factor in determining why some milk powders apparently are of poor baking quality. The presence of milk solids in a bread dough increases the bromate requirement (13, 21), absorption (25), and fermentation time (8, 9). Also there is indication that the presence of shortening may influence the baking performance of a dry milk (2, 5). The water supply should not be overlooked as it is well-known that salts have a great influence on the dough characteristics (27). The mixing time is important, some dry milks requiring very long mixing and others a relatively short mixing period for optimal results (30). The flour used is very important since optimal baking conditions for flours, which vary widely in themselves, may be modified to different extents by the presence of the milk (8, 9, 21). With these variables in mind it becomes increasingly apparent that there is a possibility that some apparently poor dry milks may be of good baking quality if the optimal conditions for their use are found. There is some feeling among test bakers that almost any milk powder can be made to produce an acceptable loaf of bread if enough time is taken to find the optimal bromate level, absorption, mixing time, salt concentration, shortening level, fermentation time, etc. for a given flour. Thus the problem may well be one of educating the milk industry to produce a fairly constant type of product under specified conditions, and of showing the baker what variables may need adjusting.

In the present investigation, it has been shown that all of the methods that have been proposed for evaluating dry milk baking quality have limitations. Some promise is shown by the use of several of these tests together. Thus if the method of manufacture can be determined by means of physical appearance, viscosity, and solubility index, the physical dough, serum protein, and reducing tests may be of considerable value in determining the extent of heat treatment the milk has undergone.

This study was undertaken as an over-all survey of the methods of evaluation now in use. The samples were manufactured under controlled conditions and 30 minute heat treatments were employed. The value of the series suffers from a lack of samples of borderline or intermediate baking quality. More work is needed critically to evaluate the ability of some of the tests to differentiate such borderline samples. Furthermore, samples manufactured with high-temperature short-time heat treatments should be studied.

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ISOLATION AND COMPOSITION OF THE SOLUBLE PENTOSANS OF WHEAT FLOURS [1]

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ABSTRACT

The water-soluble polysaccharide complex (crude pentosan) of flours prepared from various varieties of wheat has been isolated and separated into pentosan-rich and hexosan-rich fractions by acetylation and fractional precipitation. The pentosan components are shown to comprise ordered series of "true" pentosans that contain both anhydro-L-arabinose and anhydro-D-xylose residues. Each series of pentosans has a composition similar to that of the others irrespective of the nature of the flour from which it was prepared. The hexosan-rich fractions appear to consist of soluble starch or dextrins, and a galactan or pentose-galactan.

Soluble pentosans in wheat flour are known to influence dough properties and, to a lesser extent, baking quality (1, 15, 18), and are important in various technological processes (3). The chemical

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nature of these polysaccharides does not appear to be well defined. Wroblewski (24) isolated a soluble polysaccharide, from a wheat diastase preparation, which yielded L-arabinose on hydrolysis and which was referred to as an "araban." Arabinose was also identified by Freeman and Gortner (6) as a component sugar of gums isolated by extracting a number of wheat flours with half-saturated ammonium sulphate. They suggested xylose as a possible additional component, although none was identified except from one Durum gum in which, however, arabinose appeared to be absent. These gums gave negative tests for galactose and uronic anhydride and were considered to be principally arabans or xylans or mixtures of both. A soluble araban of wheat flour was also mentioned by Geoffrey (7) who found only arabinose in the hydrolysate of a levorotatory polysaccharide prepared from an aqueous extract of wheat flour. More recently, a polysaccharide extracted with dilute alcohol from wheat β-amylase was found by Ford and Peat (5) to contain D-xylose and D-galactose residues as well as L-arabinose.

All of these polysaccharides were easily soluble in water and appeared to be composed principally of anhydropentose sugar residues. The differences in composition noted may have been attributable to several causes. The variety of procedures used in isolation made it possible that a number of different polysaccharides were actually involved, each type of extraction yielding a somewhat different product. It was equally likely that difficulties in characterizing mixtures of sugars, especially for early investigators, sometimes prevented identification of all sugars present. Furthermore, starting materials varied widely, sometimes being whole flours and from different wheats, sometimes crude enzyme preparations from wheat.

From these considerations it appeared that further investigation of the chemical constitution of these soluble polysaccharides should embody isolation by mild chemical treatment of at least the major part of the soluble pentosan components obtained from different types of wheat flours. Newer techniques in sugar chemistry could then be used to obtain information more complete than heretofore recorded. In the present research, all of the polysaccharide isolated from a variety of wheat flours by extracting with water at room temperature was examined, attention being centered on the pentosan components.

Materials and Methods

Materials. Preliminary experiments on the isolation of pentosans as acetates were conducted with a commercial all-purpose household flour. The large scale extraction (Tables II and III) was made on a commercial type patent flour that was unbleached and contained no

improvers. Varietal flours used in the comparative study were experimentally milled from spring (Thatcher), durum (Carleton), and winter (Dawson's Golden Chaff) wheats.

Analytical Methods. Nitrogen was determined by the micro-Kjeldahl procedure. Furfural was estimated by the bromine oxidation method of Hughes and Acree (11). Acetyl was determined by saponifying the acetate in acetone at room temperature with an excess of 0.08 *N* sodium hydroxide and back-titrating with 0.08 *N* hydrochloric acid with phenolphthalein. Uronic anhydride was estimated by the micro-method of Tracey (21). Viscosity was measured with an Ostwald-type viscometer using solutions of the acetate in chloroform at concentrations of 0.05, 0.075, 0.1, 0.15, and 0.2%; intrinsic viscosity was evaluated by extrapolation to zero concentration of the plot of calculated values for η_{sp}/c versus concentration.

Preparation of Crude Pentosans. Flour (1,500 g.) was taken up in water (3,000 ml.) at 15°C. and the mixture agitated in a Waring Blendor for 5 minutes (1). The suspension was centrifuged and the supernatant filtered through glass wool. In a larger preparation from commercial flour the supernatant was obtained as a product of a wheat starch preparation made according to the procedure of Shewfelt and Adams (19), in which the ratio of flour to water was 1 to 4. The clear extract, obtained by either method, was adjusted to pH 3 with hydrochloric acid and made up to 70% with respect to alcohol. A voluminous white precipitate was separated in the centrifuge, washed with alcohol and ether, and dried *in vacuo* over phosphorus pentoxide and paraffin. The product was a white, coarse powder. Data for the three varietal flours are presented in Table I.

TABLE I

THE CRUDE PENTOSANS OF FLOURS MILLED FROM THREE WHEAT VARIETIES

Variety	Crude Pentosans Soluble in Water	Pentosan Content	Nitrogen
	%	%	%
Thatcher	1.17	35.3	6.96
Carleton	1.17	31.9	5.85
Dawson's Golden Chaff	1.13	25.8	8.70

Acetylation of the Crude Pentosans. The method of Carson and Maclay (2) was used. In a typical preparation crude pentosan (4 g.; furfural, 18.2%; nitrogen, 12.63%) was suspended in formamide (28 cc.) and the resultant smooth paste was mixed with pyridine (56 cc.). Acetic anhydride (28 cc.) was added with continuous stirring over a period of 1.5 hours (temperature increased to 50°C.). After standing

at room temperature for 20 hours the solution was poured into ice-water and the precipitated acetate was collected by filtration, washed with water, and dried *in vacuo* at 55°C. Re-acetylation without formamide yielded 5.3 g. of the crude acetate as a fine white powder (furfural, 13.2%; nitrogen, 1.15%; acetyl, 39.8%).

Fractionation of the Crude Acetate. The crude acetate was shaken for one to two days with 50 volumes of acetone or chloroform per gram. The acetate prepared from the commercial flour was extracted with chloroform directly, but those of the varietal flours were extracted first with acetone and then with chloroform. Light petroleum ether (boiling range 65–110°) was added to the clear extract until a definite turbidity was observed. Material which separated out was washed with petroleum ether and solvent mixed in the same proportion as that of the supernatant liquor, again taken up in solvent and reprecipitated with petroleum ether. The precipitates were washed finally with petroleum ether and dried *in vacuo* over phosphorus pentoxide and paraffin. By successive addition of petroleum ether in this manner a series of fractions was obtained which, for the commercial flour, are described in Table II and, for the three varietal flours, in Table V. Recovery of material was practically quantitative.

Refractionation, such as described in Table IV, was achieved in like manner, the fraction being first taken up in solvent and subfractions precipitated by addition of successive aliquots of petroleum ether.

Deacetylation. Barium methylate in catalytic amounts (25) was employed at the outset but was not adequately effective in removing acetyl groups; aqueous potassium hydroxide was more satisfactory and yielded deacetylated products that were lighter in color and more readily soluble in water. In deacetylation with barium methylate the acetate was suspended in 10 volumes per gram of absolute methanol, 0.5 *N* barium methylate was added to a concentration of 0.1% of theory and the suspension heated under reflux for 7 minutes. Alternatively, the acetate was shaken at room temperature with five volumes of 0.2 *N* potassium hydroxide per gram (20–25% excess) for 18 to 20 hours; sometimes the sample was dissolved in acetone prior to addition of the alkali. The reaction mixture was diluted with water to two or three times its volume, acidified to pH 2 with hydrochloric acid, and three volumes of alcohol added. Deacetylated products were recovered in the centrifuge, washed with alcohol and ether and dried *in vacuo* over phosphorus pentoxide and paraffin. In a typical experiment 1.0 g. pentosan acetate yielded 0.6 g. of deacetylated pentosan as a fine, white powder readily soluble in water.

Examination of Polysaccharide for Constituent Sugars. Deacetyl-

ated polysaccharide was hydrolyzed to constant rotation and aldose content by heating in a boiling-water bath at a concentration of 1% in 0.1 *N* sulphuric acid. Aldose was estimated by the hypoiodite method according to Rutherford *et al.* (17). Constituent sugars were identified tentatively and estimated by filter paper chromatography, described below, the identification of each being confirmed as follows: L-arabinose as the benzoylhydrazone (10), m.p. 187–188°C., mixed m.p. 186.5–187.5°C. with authentic L-arabinosebenzoylhydrazone; D-xylose as the crystalline-free sugar and D-galactose as the α-methyl-phenylhydrazone (10) by comparison of their X-ray diffraction patterns with those of authentic samples; D-glucose by selective fermentation with a strain of *Torula utilis* shown to be specific for D-glucose.

Filter Paper Chromatography. The technique employed was a composite of a number of published methods. A paper containing the sugar sample was arranged for descending chromatography in a cylindrical glass jar with ground-seal lid. One of several solvent systems was used to separate the sugars, including ethyl acetate—water with pyridine or acetic acid (12) and butanol—water with ethanol or acetic acid (13). The organic solvent-rich layer was placed in a trough from which the paper was suspended and the water-rich layer in a shallow dish at the bottom of the jar. After the solvent had irrigated the paper for the desired length of time the papers were dried at room temperature. Individual sugars were located by spraying the paper with ammoniacal silver nitrate (13) or, preferably, with aniline phthalate (14) followed by heating at 110°C. for 10 minutes. Identity of the sugars was indicated by the mobility as compared with that of known sugars chromatogrammed concurrently on the same paper strip. Of the solvent systems examined the combination ethyl acetate-pyridine-water (2:1:2) was most useful for the mixtures of sugars encountered and was generally used in effecting adequate separation of individual sugars for subsequent estimation.

Sugars separated in the manner described were estimated by a procedure similar to those of Flood *et al.* (4) and of Hawthorne (8). A measured volume of the hydrolysate (the concentration of acid employed did not affect sugar separations and hence the hydrolysate was not neutralized) was added to the paper, development of the chromatogram was allowed to proceed as usual, and the papers were dried at room temperature for at least 5 hours. Narrow strips were cut lengthwise from both edges of the paper and the position of the sugars located with the indicator. Sections of the unsprayed paper remaining were cut to correspond to areas occupied by individual sugars, together with a section of equal area containing no sugar which served as a blank.

The paper sections were extracted by being suspended each in a tube where it was close to the surface of water under gentle reflux. Reducing sugar content of each extract was estimated iodometrically with the micro Somogyi reagent (20), standard solutions of the known sugars in similar concentration being used concurrently for comparison. Recovery of sugars was generally in excess of 90% of theory based on the original weight of polysaccharide. Results of these estimations are presented in Tables III, IV, VI, and VII.

Results and Discussion

Commercial Flours. The crude pentosans extractable at room temperature contained 30 to 35% pentosan and 15 to 20% protein. Acetylation eliminated up to 50% of the protein and a further 25 to 30% was separated by extraction of the crude acetates. Fractional precipitation from the extracts yielded the series of purified polysaccharides described in Table II. Fractions 1 and 2 contained some

TABLE II

FRACTIONATION OF ACETYLATED CRUDE POLYSACCHARIDE FROM COMMERCIAL PATENT FLOUR

Fraction	Weight[1]	$[\alpha]_D^{25}$[2]	Furfural	Acetyl	Nitrogen
	g.		%	%	%
1	3.5	—	4.1	—	7.8
2	15.0	—	20.2	—	4.2
3	28.1	−90	29.6	38.6	1.0
4	41.7	+153	0	45.1	0.02
5	3.9	+150	0	45.0	0.05
6	0.8	+122	—	44.8	—

[1] From 95.0 g. crude acetate.
[2] Concn., 0.6% in chloroform.

pentosan but were high in nitrogen as well; reprecipitation of Fraction 2 reduced the nitrogen content to 0.7% and yielded a small subfraction designated as 2A. Fraction 3 gave analyses close to those for a pentosan diacetate (calc. for araban diacetate: furfural, 32.0%; acetyl, 39.9%). On the other hand, Fractions 4, 5, and 6 contained no pentosan and had specific rotation and acetyl values similar to those of starch acetates. The deacetylated polysaccharides on hydrolysis yielded the sugars listed in Table III. Both L-arabinose and D-xylose were found, associated with small amounts of D-galactose and D-glucose. Fractions 4, 5, and 6 contained only D-glucose and in their reaction with iodine agreed with the starch or dextrin-like properties previously indicated; these fractions were not examined further.

The presence of glucose in Fractions 2 and 3 suggested that traces of the more soluble hexosans may have been occluded by the pentosan

TABLE III

COMPOSITION OF THE POLYSACCHARIDE FRACTIONS AFTER DEACETYLATION

Fraction	$[\alpha]_D^{25}$ [1]		Sugars			
	Polymer	Hydrolysate	Arabinose	Xylose	Galactose	Glucose
			%	%	%	%
2	−100	—	35.0	54.8	6.4	3.0
3	−110	—	36.3	57.1	3.6	3.0
2A	+122	+61.0	4.8	6.1	5.0	84.0
4	—	+55.5	—	—	—	100.0
5	+165	+54.0	—	—	—	100.0
6	+187	+53.8	—	—	—	100.0

[1] Concn., 1.0% in 0.5 *N* H_2SO_4.

particles during precipitation. A careful refractionation of Fraction 3-acetate, results of which are presented in Table IV, concentrated the glucose and also the galactose almost entirely into the tail fraction (Fraction 36). A similar separation of all of the galactose and glucose, still as components of intact polysaccharides, from the major portion of the pentosan, was achieved by a gentle graded hydrolysis described in detail in a succeeding communication (16). This ready segregation of pentosan from hexosan by purely physical or mild chemical means

TABLE IV

REFRACTIONATION OF POLYSACCHARIDE "3" ACETATE

Fraction[1]	Weight[2]	$[\alpha]_D^{25}$ [3]	Sugars in Deacetylated Material			
			Arabinose	Xylose	Galactose	Glucose
	g.		%	%	%	%
31	0.20	—	—	—	—	—
32	0.15	—	—	—	—	—
33	1.11	−108.7	36.6	62.2	1.0	trace
34	1.50	−110.0	—	—	—	—
35	1.02	−107.5	46.0	52.2	1.2	0.8
36	0.15	+ 9.8	16.4	18.0	27.0	38.6

[1] Code numbers refer to sub-fractions of polysaccharide 3-acetate.
[2] From 4.0 g. sample.
[3] Concn., 0.4% in chloroform.

strongly indicated that "true" pentosans were present in the water extract of wheat flour. The pentosans appeared to be neither araban nor xylan exclusively but composed of both anhydro-L-arabinose and anhydro-D-xylose units.

It was possible that traces of uronic acid were associated with these pentosans either as part of the molecule or as impurities. For example, Fraction 3 (Table III) yielded carbon dioxide, uncorrected for that

evolved by non-uronide material, equivalent to 5.5% uronic anhydride. Refractionation appreciably lowered this apparent uronic anhydride content, Subfractions 3 and 5 (Table IV) each giving yields of carbon dioxide, again uncorrected, equivalent to 3 to 4% uronic anhydride. The degree of accuracy of the analytical methods and unsuitability of non-uronide blank corrections for these materials made questionable the interpretation that uronic anhydride units were actually present as part of the pentosan molecules. Therefore continued use of the term "pentosan" appeared to be justified since the polysaccharides were composed of anhydropentose residues to the extent of at least 95%.

TABLE V

FRACTIONATION OF CRUDE POLYSACCHARIDE ACETATES FROM THREE VARIETIES OF WHEAT FLOUR

Variety	Fraction	Weight	$[\alpha]_D^{25}$[1]	Acetyl	Nitrogen
		g.		%	%
Thatcher (Spring)	A 1	3.1	−101.5	38.8	0.70
	A 2	0.5	−100.0	38.1	0.18
	A 3	0.3	−108.2	37.0	0.13
	A 4	1.7	+ 36.5	39.4	0.24
	A 5	0.4	00.0	42.0	0.50
	A 6	0.9	—	—	—
Carleton (Durum)	B 1	3.3	85.4	38.1	0.91
	B 2	2.3	− 46.6	38.4	0.90
	B 3	0.9	+ 36.3	41.2	0.48
	B 4	2.2	+110.0	41.7	0.25
	B 5	0.7	+138.3	42.3	0.12
Dawson's Golden Chaff (Winter)	C 1	2.0	− 95.5	38.2	1.08
	C 2	0.8	−108.8	38.8	0.43
	C 3	0.4	− 88.8	39.2	0.40
	C 4	1.4	−100.2	38.5	0.31
	C 5	2.1	0.0	40.8	0.36

[1] Concn., 0.5% in acetone.

Non-commercial Flours. As previously noted, the literature suggested that different flours might be expected to yield pentosans having widely different chemical characteristics. The possibility was of interest especially for flours showing widely divergent baking properties, which are generally attributed to variations in protein quality. Since pentosans are known to affect physical characteristics of flour they might constitute an additional factor in determining these differences. On the other hand, like starch, they might be chemically similar irrespective of source. As an approach to this problem the acetylation procedure, which had effectively isolated pentosan from the hexosan and protein extracted simultaneously from the commercial flour was applied to a number of different flours. Three flours were

selected to represent three broad classes of wheats—spring, durum, and winter. Table V describes the fractions precipitated from the respective acetone extracts of the three acetates, and Table VI presents the component sugars in the major fractions.

TABLE VI

COMPONENT SUGARS OF MAIN FRACTIONS OF WATER EXTRACT FROM THREE FLOURS

Variety	Fraction	Sugars			
		Arabinose	Xylose	Galactose	Glucose
		%	%	%	%
Thatcher (Spring)	A 1	31.0	60.6	5.4	3.0
	A 2	40.0	60.0	—	—
	A 4	42.8	22.6	35.0[1]	
Carleton (Durum)	B 1	28.9	62.3	6.3	2.5
	B 2	36.0	34.0	17.5	12.4
	B 4	9.5	3.0	87.5[1]	
Dawson's Golden Chaff (Winter)	C 1	35.2	53.7	9.8	1.0
	C 4	42.1	53.2	4.6	trace
	C 5	37.6	15.5	43.1[1]	

[1] Galactose plus glucose.

Precipitations from solution in acetone with petroleum ether of necessity made for an empirical procedure. All three extracts, nonetheless, responded to this treatment with considerable uniformity. A gradation of composition was indicated in Table V ranging from predominantly pentosan in the earlier fractions to predominantly hexosan in the later more soluble fractions, in a manner similar to that described previously (Table II). This trend was emphasized by analysis of the deacetylated polysaccharides (Table VI). Further analogies were seen between the groups of fractions in that all contained both L-arabinose and D-xylose residues accompanied by smaller amounts of D-galactose and D-glucose. The major pentosan Fractions A 1, B 1 and C 1, were closely similar in their contents of these sugars and similar also to the main pentosan fraction of the commercial patent flour (Table III). The second main Fractions, A 2, B 2, and C 4, were also reasonably uniform in composition. Like the pentosans of the commercial flour, the apparent uronic anhydride content was low in these fractions. Fractions A 1, B 1, and C 4, chosen as representative samples, yielded carbon dioxide, uncorrected for non-uronide, equivalent to 3.4, 3.5, and 3.1% uronic anhydride, respectively. Unlike the commercial flour, no fractions derived from these flours consisted entirely of glucose, although the latter fractions all contained a high proportion of hexosan.

Further comparisons could be drawn between the soluble polysaccharides of the three flours from additional fractions, described in Table VII isolated by extracting the acetone-insoluble acetate residue of each with chloroform. These fractions had similar composition and were unique in containing a high percentage of galactose and no glucose. The complete absence of glucose in these as well as in

TABLE VII

COMPOSITION OF ACETONE-INSOLUBLE, CHLOROFORM-SOLUBLE POLYSACCHARIDE ACETATES

Variety	Sample Weight	Nitrogen	Sugars in Hydrolysate		
			Arabinose	Xylose	Galactose
	g.	%	%	%	%
Thatcher (Spring)	1.68	2.59	42.1	25.6	32.2
Carleton (Durum)	0.70	3.75	38.6	33.1	28.4
Dawson's Golden Chaff (Winter)	0.75	2.27	41.2	26.1	32.8

Fractions A 2 and C 4 (Table VI), indicated clearly the existence of a glucose polymer distinct from the galactose component. Previously it had been demonstrated that the hexosan was independent of the major part of the pentosan. The present indication that the glucose and galactose were also unrelated as polysaccharide constituent sugars suggested that wheat flours contain a soluble galactan. Since, however, galactose did not occur in any fraction completely free of pentose it is possible that some pentose residues may be associated with the galactose to form a "pentosan-galactan." An arabo-galactan from larch wood was described by Wise and Peterson (23) and by White (22), and the galactan of pectin was reviewed by Hirst and Jones (9). The very small quantities of the galactose component involved in the present work has precluded its isolation, and no relation to the other galactans may be drawn at this time.

All of the foregoing results indicated a good deal of similarity in composition between the soluble pentosans of the three flours. There appeared to be some difference, however, in molecular size of the major pentosan fractions. The acetates of Fractions A 1, B 1, and C 1 gave intrinsic viscosities in chloroform of 3.4, 1.9, and 3.4, respectively. The lower intrinsic viscosity of the Durum pentosan may have been related to degradation of polysaccharide during the milling process, since this flour yielded considerably more soluble hexosan than did the other two flours (Table VI). In support of this suggestion it may be noted that the commercial patent flour also had a high content of solu-

ble starch, and the acetates of its Fractions 33 and 35 (Table IV) in chloroform showed intrinsic viscosities of 1.8 and 2.0, respectively.

When the proportions of arabinose to xylose for the various fractions described in Table VI were considered, an ordered gradation of values was found throughout. This is shown in Table VIII. Arabinose to xylose ratios for several fractions of the commercial flour (Table III) are included to illustrate this trend further. It was seen that the relative amounts of arabinose always increased regularly from the less to the more soluble fractions, the change being of the same order in the fractions of the three varieties of flours, but more pronounced than for the commercial patent flour. These variations in the ratio of arabinose to xylose together with the similarities noted previously were taken to indicate that the soluble pentosans of wheat flours comprise a series of arabinose-xylose polysaccharides, having varied composition and structure within the series. However the groups of pentosans appeared to be of similar composition irrespective of the nature of the flour from which they were isolated.

TABLE VIII

RATIOS OF ARABINOSE TO XYLOSE IN DIFFERENT FRACTIONS

Sample	Fraction	Arabinose:Xylose
Commercial	2	1:1.6
	2 A	1:1.3
	33	1:1.7
	35	1:1.1
	36	1:1.0
Thatcher (Spring)	A 1	1:2
	A 2	1:1.5
	A 4	2:1
Carleton (Durum)	B 1	1:2
	B 2	1:1
	B 4	3:1
Dawson's Golden Chaff (Winter)	C 1	1:1.5
	C 4	1:1.2
	C 5	2.4:1

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STRUCTURE OF THE SOLUBLE PENTOSANS OF WHEAT FLOURS [1]

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ABSTRACT

Graded hydrolysis of the pentosans effected preferential removal of anhydro-L-arabinose units, leading to formation of an insoluble residue composed principally of anhydro-D-xylose units. Methylation analysis of the pentosans yielded 2,3,5-trimethyl-L-arabofuranose (3 moles), 2,3-dimethyl-D-xylose (3 moles), 2-methyl-D-xylose (1 mole), and D-xylose (1 mole). Periodate oxidation coupled with graded hydrolysis was employed to choose between the different possible structural types. A basic repeating unit for the major portion of the soluble pentosans in wheat flours is proposed: The chief characteristics are a straight chain of anhydro-D-xylose residues linked β-1, 4-, to which are appended single units of anhydro-L-arabofuranose through 1,2- and 1,3-linkages, making for a highly-branched structure. Analogy is drawn between this structure and that of known pentosans of woods and straws to suggest a solubility series among these types of polysaccharides in general. According to this scheme the xylan components may become more soluble or less soluble as the incidence of arabinose side-chains increases or decreases.

A previous communication (14) described the isolation of pentosans composed of L-arabinose and D-xylose from the water extract of various wheat flours. Ford and Peat (7) found a water-soluble polysaccharide associated with β-amylase of wheat flour, which contained these sugars and in addition D-galactose, and for which a structure was proposed on the basis of methylation data. This polysaccharide most probably was related to the present pentosans but since the latter were separable from a galactose-rich polysaccharide by repeated fractionation of their acetates (14) it appeared desirable to investigate further the structure of the soluble pentosans. In the present instance, graded hydrolysis and periodate oxidation together with a re-examination of the methylation data indicated a structure somewhat different from that proposed by Ford and Peat.

Materials and Methods

The pentosans, designated "3", A_1, B_1 and C_1, were the major pentosan fractions prepared from a commercial patent flour "3" and flours representing spring (A_1), durum (B_1), and winter (C_1) wheats,

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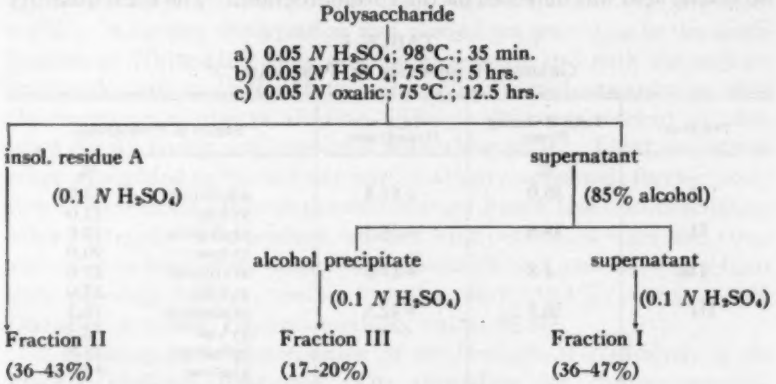
respectively, and are described in detail in a previous communication (14). Total reducing sugar was estimated by hypoiodite oxidation (17). The general procedure for analysis of sugars by paper chromatography is given in a previous communication (14); modifications necessary for the analysis of mixtures of methylated sugars are noted below.

Graded Hydrolysis. Graded hydrolysis was carried out on the pentosans according to the general scheme shown in Fig. 1. In a



Fraction I —contained at least 75% of the arabinose.
Fraction II —composition was at least 90% xylose.
Fraction III—contained the galactose and glucose components together with the remainder of the xylose.

FIG. 1. Graded hydrolysis of Pentosan "3."

typical experiment, 0.99 g. of pentosan "3" was digested with 150 ml. of 0.05 *N* sulfuric acid at 75°–77° for 4.5 hours. The solution was cooled to 0° and the white precipitate formed (insoluble residue A) was centrifuged and washed with water. Alcohol was added to the supernatant liquor at the outset, causing precipitation of a small flocculent precipitate, not indicated in Fig. 1, which is described below. A further precipitation was achieved by concentrating the supernatant liquor *in vacuo* on a steam-bath to a volume of 30 ml. and adding alcohol to a concentration of 85% (alcohol precipitate). The final supernatant liquor was concentrated *in vacuo* on a steam-bath to remove alcohol, the concentration of sulfuric acid was adjusted to 0.1 *N*, and hydrolysis resumed on a boiling-water bath to constant rotation (Fraction I). The insoluble reside A, small precipitate, and alcohol precipitate were each taken up in 0.1 *N* sulfuric acid and hydrolyzed on a boiling-water bath to constant rotation, their hydrolysates corresponding to Fractions II, IIa, and III, respectively. Total reducing

sugar was estimated on each fraction, and aliquots of each were examined qualitatively and quantitatively by paper chromatography for constituent sugars. Results are summarized in Table I. Fraction IIa, being almost identical with II and of small proportion, was included as part of the latter in Fig. 1. A small amount of material in Fraction II remained insoluble after prolonged hydrolysis. It gave a positive naphthoresorcinol test for uronic acid. On hydrolysis with 4 *N* sulfuric acid at 60° for seven days it yielded a trace of xylose but no uronic acid was detected on the chromatogram. The total quantity

TABLE I

GRADED HYDROLYSIS OF PENTOSAN "3"

Fraction	Total Reducing Power	$[\alpha]_D$ of Hydrolysate	Sugars in Hydrolysate	
	%			%
I	36.0	+81.8	arabinose	83.0
			xylose	17.0
II	38.6	+18.5	arabinose	10.0
			xylose	90.0
IIa	4.8	+17.6	arabinose	17.0
			xylose	83.0
III	20.5	+42.5	arabinose	10.3
			xylose	52.5
			galactose	23.8
			glucose	13.3

of sugar accounted for in Table I was 0.89 g. which, corrected for the unhydrolyzed residue and the protein content of the sample, amounted to 90% of the weight of the original pentosan sample. In graded hydrolysis of pentosans A_1, B_1, and C_1, the results of which are presented in Table II, 0.1 *N* sulfuric acid, rather than 0.05 *N*, was used

TABLE II

GRADED HYDROLYSIS OF PENTOSANS A_1, B_1, C_1

(According to scheme in Fig. 1)

	% of total			Sugars found		
	A_1	B_1	C_1	A_1	B_1	C_1
Fraction I	20.2	27.0	22.0	L-arabinose; D-xylose (trace)		
Fraction II	25.5	21.5	28.0	D-xylose; L-arabinose (trace)		
Fraction III	54.0	51.4	49.8	D-xylose; L-arabinose (trace)		
				D-galactose (moderate); D-glucose (moderate)		

in the preliminary hydrolysis and probably accounts for the increase in the proportion of material recovered as "alcohol precipitate."

Methylation of Pentosan 3. Pentosan "3" (1.12 g.), reprecipitated to reduce the hexosan content to approximately 4%, was pasted with 15 ml. of water and the suspension was diluted with 13.5 ml. of 40%

sodium hydroxide. Methyl sulfate (10 ml.) was added under an atmosphere of nitrogen during a period of 3 hours, accompanied by stirring that was continued for a further 15 hours, acetone being added to diminish foaming. The reaction mixture was diluted with water, neutralized in the cold with 25% sulfuric acid, and concentrated *in vacuo* to a thick paste. After two further methylations the final concentrate was extracted continuously with chloroform. Distillation of the solvent left a syrup, which was twice methylated as above and again extracted with chloroform (methyoxy value, 36.4%; nitrogen, 0.1%). A further methylation was carried out according to the modification of White (18) using acetone as solvent and with the sodium hydroxide and methyl sulfate being added simultaneously so that the reaction was always alkaline. The product was worked up, dialysed for 48 hours, and extracted with chloroform. Light petroleum ether was added to the extract until turbidity appeared; further addition of petroleum ether to the supernatant liquor failed to precipitate other material. The product, washed with petroleum ether and ether and dried *in vacuo* over phosphorus pentoxide and paraffin, was a light grey powder, 0.83 g.; methoxy value, 38.7, 38.4%; nitrogen, nil. Dimethyl pentosan requires methoxy value, 38.8%.

Chromatographic Examination of the Products of Hydrolysis of the Fully Methylated Pentosans. The procedure for chromatographic examination was similar to that described by Hirst *et al.* (13). Samples of the methylated pentosans (50 mg.) were heated at 100°C., each in a sealed tube with 1.5 ml. of 4% methanolic hydrogen chloride for 5, 10.5 and 16 hours, respectively, and then in the opened tube with normal hydrochloric acid for 5, 4.5 and 3 hours, respectively. The hydrolysates were neutralized with silver carbonate, excess silver being removed with hydrogen sulfide, and were examined by paper partition chromatography. Four distinct compounds were found in each hydrolysate using two solvent systems: butanol-ethanol-water-ammonia (13), and methyl ethyl ketone-water (2). In both solvents and with 2,3,5-trimethyl-L-arabofuranose, 2,3-dimethyl-D-xylose, and D-xylose as reference sugars, the fastest-moving spot corresponded to the trimethyl arabinose, the second to the dimethyl xylose, the third to a monomethyl pentose, and the slowest to D-xylose. This was in agreement with the four pentose derivatives found by Ford and Peat (7). A two-dimensional chromatogram using the same two solvents consecutively failed to indicate the presence of compounds other than these four. Quantitative chromatographic analysis of the three hydrolysates, using hypoidite according to Hirst *et al.* (13) for estimation of the sugars separated on the chromatogram and with D-xylose as the reference sugar, gave results in close agreement. The average molar

quantities were: trimethyl, 2.8 moles; dimethyl, 3.4 moles; monomethyl, 1 mole; xylose, 1 mole; or in the ratio of approximately 3:3:1:1.

Methyl-2,3,5-trimethyl-L-arabofuranoside. Methylated pentosan, 0.6 g., was heated at 100°C. in a sealed tube with 4% methanolic hydrogen chloride for 12 hours. Hydrogen chloride was neutralized with silver carbonate, and the alcohol was distilled to yield a brown oil, 0.73 g. The oil was taken up in water and continuously extracted with light petroleum ether (b.p. 36–37.5°C.) to remove fully methylated glycoside (3). Distillation of the petroleum ether solution yielded a pale yellow oil which proved to be methyl-2,3,5-trimethyl-L-arabofuranoside. Total yield from two extractions, 0.24 g.; η_D^{25}, 1.4325; methoxy value, 59.0% (required, 60.2%); readily decolorized neutral permanganate; $[\alpha]_D^{25}$, −78.3°, changing to a final value of $[\alpha]_D^{25}$, −45.1° on hydrolysis with *N*/25 hydrobromic acid (concn., 1.75%) (7). A portion of the methylated glycosides remaining in the aqueous layer was hydrolyzed with normal hydrochloric acid and examined on a paper chromatogram. It was found that the spot corresponding to the fully methylated glycoside had been completely removed by the extraction with petroleum ether, amounting to approximately 35 moles per cent of the total. Since this figure was equivalent as well to the arabinose content of the unmethylated pentosan, it followed that the remaining three compounds were derived from anhydro-xylose units.

2,3-dimethyl-D-xylose. The aqueous solution remaining from the petroleum ether extraction was extracted continuously with chloroform until a trace of the monomethyl derivative began to appear in the extract, as shown by chromatographic examination of acid-hydrolyzed aliquots of the extract. Distillation of the chloroform yielded a pale yellow oil, 0.25 g. This was taken up in normal hydrochloric acid and heated at 100°C. for 11 hours. Silver carbonate was added to neutrality and the aqueous residue was extracted with chloroform to yield 2,3-dimethyl-D-xylose; η_D^{21}, 1.4685; $[\alpha]_D^{27}$, +26.0 (concn., 0.85% in water); methoxy value, 32.8% (required, 34.8%). The anilide, prepared by refluxing an alcoholic solution of the product with aniline, was twice recrystallized from ethyl acetate containing a few per cent of petroleum ether. M.p. 122°–124°C., underpressed by admixture with authentic 2,3-dimethyl-D-xylose anilide, m.p. 124–126°C.; the X-ray diffraction diagrams of the unknown and known anilides were identical.

Monomethyl-D-xylose. Following extraction of the dimethyl glycoside, the aqueous layer was concentrated *in vacuo* to small volume, adjusted to 1.0 *N* with respect to hydrochloric acid, and heated at 100°C. for 4.5 hours. The hydrolysate was neutralized with silver carbonate and concentrated *in vacuo* to a syrup which was extracted with acetone; the acetone was distilled and the residue was taken up in

a few ml. of water. Chromatographic analysis showed the presence of monomethyl xylose, 63.5%; D-xylose, 24.6%; and dimethyl xylose, 12.1%. Aliquots of the solution were concentrated to dryness and the methoxyl content estimated. Found, methoxy value, 17.9%. Calculated for monomethyl xylose contaminated with xylose and dimethyl xylose in the amounts noted, methoxy value, 16.2%. The solution remaining was chromatogrammed to yield an extract containing 7.6 mg. of the pure monomethyl xylose. Found $[\alpha]_D^{27}$, +35.3°; (concn., 0.17% in water) 2-methyl-D-xylose gives $[\alpha]_D$, +35.9° (16). The acetone-insoluble reside contained D-xylose and a trace of the monomethyl derivative.

An attempt to locate the position of the methoxyl group was made using paper partition chromatography combined with periodate oxidation. Three spots of the original hydrolysate, which contained all four compounds as the free sugars, were placed along the starting line on a paper strip. Two of the spots were sprayed with 2% aqueous sodium periodate (pH 4), the paper heated for 5 minutes at 60°C. in an atmosphere of nitrogen, and the treatment was repeated. The two spots were then sprayed with 20% aqueous ethylene glycol to remove excess periodate and the paper was dried at room temperature in an atmosphere of nitrogen for 10 minutes. This procedure was similar to that of Buchanan *et al.* for the detection of glycosides (5). The sugars were chromatogrammed in the usual manner, and their positions were located with an aniline phthalate spray (14). Comparison of the sugars separated from the treated and untreated spots showed that xylose and monomethyl xylose had been oxidized, whereas the dimethyl xylose and trimethyl arabinose were unaffected. A second paper strip, on which the hydrolysate had been chromatogrammed in the usual manner without preliminary oxidation, was treated with the periodate and glycol sprays as described above. Then followed a spray with 5% aqueous potassium iodide as described by Buchanan *et al.* for the detection of formic acid (5). An iodine stain indicative of formic acid appeared only in the position corresponding to xylose. 3- or 4-methyl xylose would be expected to yield formic acid readily on oxdiation with periodate. However, 2-methyl xylose could yield formic acid only through mutarotation, which might have been inappreciable under the conditions of the oxidation. The latter was further indicated by the fact that 2,3-dimethyl xylose was not oxidized even after three successive applications of the periodate spray, which would be expected only if no mutarotation had occurred.

Oxidation with Periodate. In a typical oxidation, pentosan "3" (0.10 g.) was dissolved in periodic acid solution (1.15 g. of periodic acid in 100 cc. of water and neutralized to methyl red with sodium hydrox-

ide) in a low actinic glassware flask (12) and the reaction temperature was maintained at 16°C. Analyses were carried out at various time intervals for periodate consumption (8) and for formic acid formation (9). Moles of periodate comsumed per pentose unit at 42 hours, 90 hours, 140 hours, and 190 hours were 0.62, 0.67, 0.73, and 0.72, respectively. After 190 hours the yield of formic acid was 0.01 moles per sugar unit, giving a corrected value for periodate consumption of 0.71 moles per mole.

The oxidized product was isolated in a separate experiment by adding an excess of glycol and six volumes of ethanol. The precipitated material was collected, taken up in water and dialysed against water until the test for iodate ion was negative. Dilution with ethanol yielded a precipitate which was collected, washed with ethanol and dried (0.037 g.). Hydrolysis with 0.1 *N* sulfuric acid followed by paper partition chromatography showed that xylose was the major constituent whereas galactose was present in small amount and a trace of arabinose was discernible.

Pentosans having diminished arabinose content were prepared for oxidation by graded hydrolysis. A sample with approximately one-half of the original arabinose content was obtained in the following manner: pentosan 3 (1.155 g.) was heated at 95° for 45 minutes with 0.025 *N* sulfuric acid. The hydrolysate was poured into a large volume of alcohol, yielding a fine white flocculus that was washed with alcohol and ether and dried *in vacuo* over phosphorus pentoxide and paraffin (wt., 0.101 g.). Only arabinose, amounting to 0.030 g., was found in the supernatant liquor, or approximately one-half of the original arabinose content (0.058 g.). Moles of periodate consumed per pentose unit by the precipitate at 24 hours, 48 hours, and 120 hours, were 0.62, 0.79, and 0.82, respectively. At 120 hours the yield of formic acid was 0.06 moles, giving a corrected value for periodate consumed of 0.76 moles per mole. Xylan containing 7% of arabinose was prepared as described previously under "Graded Hydrolysis." Since it was insoluble in the periodate reagent the suspension was shaken throughout the oxidation period. Moles of periodate consumed per pentose unit at 120 hours, 192 hours, and 360 hours were 0.85, 0.94, and 1.03, respectively. At 360 hours the yield of formic acid was 0.09 moles, giving a corrected value for periodate consumed of 0.94 moles per mole.

Preparation of "Xylan" by Graded Hydrolysis of Crude Pentosans. Thatcher flour was extracted with water and the crude pentosans were precipitated by addition of alcohol as previously described (14). Crude pentosan, 15.0 g., was taken up in 1 l. of water and the solution was clarified by centrifuging. Sulfuric acid was added to a con-

centration of 0.05 *N* and the solution was heated at 92°C. for one hour. A fine white precipitate that formed as the solution cooled was recovered on the centrifuge, washed with alcohol and ether and dried *in vacuo* over phosphorus pentoxide and paraffin. Wt., 1.35 g.; nitrogen 0.46%. A portion of the product was suspended in 3% nitric acid and heated under gentle reflux for one hour, according to the procedure of Hampton *et al.* for hydrolysis of xylan (10). Chromatographic examination showed the presence of xylose and only a trace of arabinose; recovery, estimated as xylose and based on rotation of the hydrolysate, was 107%. The rotation of the pentosan was $[\alpha]_D^{26}$ −100° (c, 1.25 in 4% NaOH) as compared with that of xylan $[\alpha]_D^{23}$ −109.5° (10).

Results and Discussion

Graded Hydrolysis. Graded hydrolysis of pentosan "3" (Fig. 1) readily liberated three quarters or more of the arabinose units, presumably having the labile furanoside configuration, at which point 80 to 90% of the xylose residues, presumably of the more resistant pyranoside type, were no longer soluble. The pentosan was indicated to consist of a core of xylose units subtended by radiating side-chains comprised of arabinose units. The branched chain permitted ready dispersion in water; removal of the arabinose side-chains, however, left the xylose core as a relatively straight-chain, and hence less soluble, residue.

The galactose and glucose components did not readily fit the pattern. They could not have formed part of a building unit in the *main* pentosan nucleus since they were readily segregated while still part of relatively large molecules. This was viewed as evidence, in support of that presented previously (14), that true pentosans are to be found in the water extract of wheat flours.

Similar response to graded hydrolysis was given by pentosans A_1, B_1, and C_1, (Table I)—easy removal of arabinose leading to precipitation of a xylan, and segregation of the hexosan components from the major portion of the pentosans. It was previously shown that these soluble pentosans have similar composition (14). Graded hydrolysis now indicated that their structures are also analogous. It appeared that further information concerning the structure of any of the four pentosans would be applicable in a general way to all four.

Methylation. Methylation studies on pentosan "3" demonstrated the presence of 2,3,5-trimethyl-L-arabofuranose, 2,3-dimethyl-D-xylose, 2-methyl-D-xylose (tentatively) and D-xylose in molar ratios of approximately 3:3:1:1, respectively. Ford and Peat (7) had identified the same products in molar ratios of 6:6:1:1 and in addition one

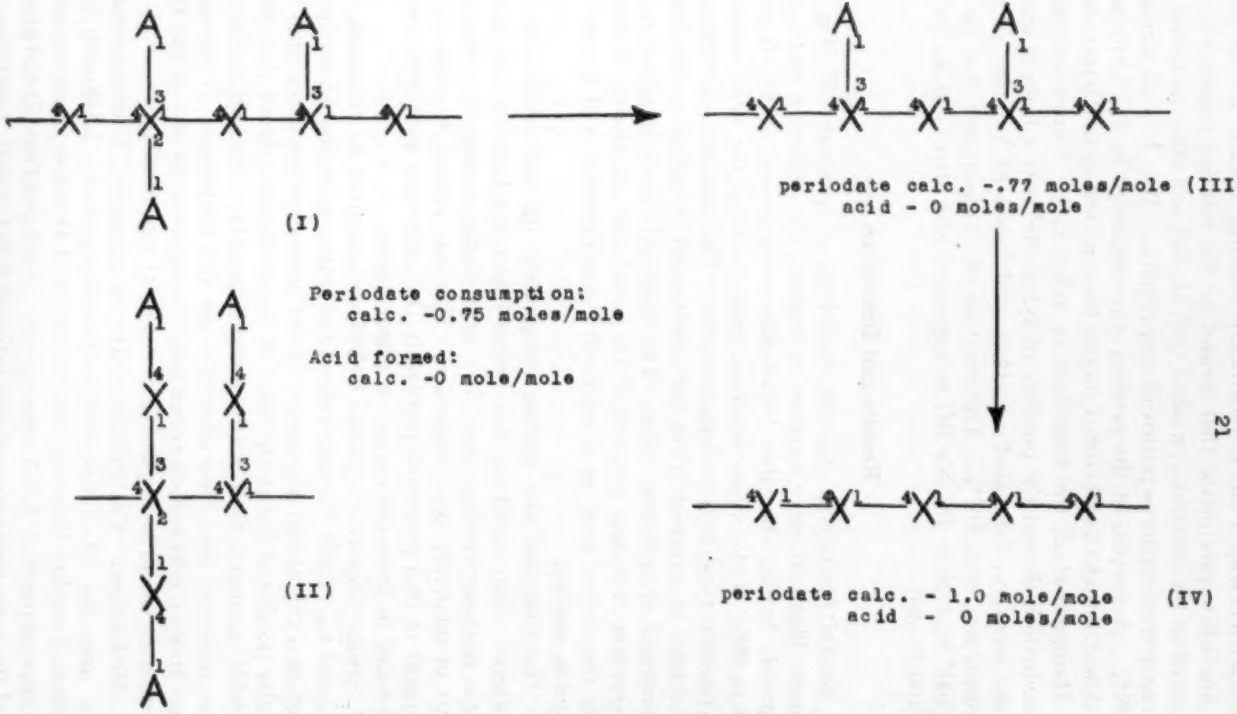


FIG. 2. Oxidation of Pentosan "3" and its products of graded hydrolyses with periodate.

mole of 2,4-dimethyl-D-galactose. These variations in results have led to the proposal of a new structure for the soluble pentosans. The basic repeating units as represented in schematic structures I and II (Fig. 2) and a combination of the two are consistent with the results from the instant methylation studies.

Periodate Oxidation. A choice between these possible structures was provided by periodate oxidation of the pentosan and products of its partial hydrolysis. Structures I and II theroetically should consume 0.75 moles of periodate per mole and yield no formic acid (except for acid derived from ends of chains, which would be very small for a large molecule). In reasonable agreement with this each of the pentosans consumed approximately 0.7 moles per mole with generation of 0.01 to 0.02 moles of formic acid per mole. When pentosan "3", with one-half of its arabinose units preferentially removed by graded hydrolysis, was oxidized the yield of formic acid increased only to 0.06 moles per mole and the periodate consumed to 0.76 moles per mole. This was in agreement with III, derived from I. Under the same conditions the xylopyranoside units in the side-chains of II would have been expected to behave as end-groups, and to result in a yield of 0.23 moles of formic acid per mole with an accompanying periodate consumption of 0.92 moles per mole.

Removal of all but 7% of the arabinose units resulted in a small increase in the yield of formic acid to 0.09 moles per mole and a periodate consumption of 0.94 moles per mole. This was consistent with IV, derived from I by complete removal of the arabinose units. The corresponding xylan core derived from II would have been expected to yield 0.6 moles of formic acid per mole and consume 1.2 moles of periodate per mole. Were the xylose residues in the side-chains of II furanoid rather than pyranoid, the graded removal of arabinose would have yielded a limiting value of 0.6 moles of periodate consumed per mole, far short of the 0.94 moles per mole observed. It appeared, therefore, that few if any xylose residues were included in side-chains. The small increasing yields of formic acid with continuing graded hydrolysis may have originated in moderate degradation of xylose chains, or also in removal of arabinose units from ends of chains thereby exposing xylopyranoside units as end groups.

Based on all three approaches—graded hydrolysis, methylation, and periodate oxidation—structure I appeared closely to represent the basic repeating unit of the pentosans. The main chain consisted of anhydro-D-xylopyranose units, linked 1, 4-, which because of the high negative rotation were probably of the β-type. From this chain radiated anhydro-L-arabofuranose residues attached at the 2- or 3-positions of individual anhydroxylose units, the latter subtending

sometimes one side chain, sometimes two. Obviously both the 2- and 3- positions were involved so as to give unmethylated D-xylose from the methylated pentosan, but the 3- position was tentatively the chief point of attachment. An indication of the number of such repeating units per molecular chain was obtained from the quantity of formic acid generated by periodate oxidation. The yields of acid, noted previously, were generally equivalent to about 0.01 moles per mole. Assuming one mole of acid per non-reducing end-group and two per reducing end-group (4, 15), these values gave an average degree of polymerization of approximately 300 corresponding to approximately 60 such repeating units (I) per molecular chain.

It was indicated previously that the pentosans comprise a series of polysaccharides having variable composition relative to their arabinose and xylose contents (14). Small amounts of pentosan having arabinose to xylose ratios markedly different from those considered here were present in fractions rich in hexosans, and may be found to be of different constitution as well. The structural arrangement presented herein, therefore, is to be regarded as a possible average building unit of the major portion of the soluble pentosans in wheat flours.

In some respects this structure closely resembles that of pentosans isolated from other sources. The xylans of some woods and straws have been shown to consist essentially of D-xylopyranose residues linked by 1,4-β-glycosidic bonds and associated with a few per cent of arabofuranose units, (10, 11) or free of arabinose (6). These xylans are sparingly soluble in water, and more soluble in dilute sodium hydroxide. Other pentosan fractions have been isolated from wheat straw that are soluble in hot water or dilute carbonate; they are predominantly xylans with 5–10% uronic anhydride, but contain as well 12–19% arabinose with appears, again, to be furanoid (1). In the present instance, with 35% or more arabinose, the pentosans obtained were soluble in cold water. All these data suggest a solubility series of xylans in which the solubility increases with increasing arabinose content. Reversal of the above order by gradual removal of these arabinose units from the soluble pentosan by graded hydrolysis yielded a decreasingly soluble core increasingly rich in xylose The cleavage proceeded to the point where substantially a pure xylan remained that was sparingly soluble in alkali, and that was chemically akin to the known xylans of wood and straw.

These observations suggest that, by linking more or fewer arabinose side-chains to a xylan, the plant may possess a pathway for synthesizing more soluble or less soluble pentosans. It is conceivable, in a wheat plant, for example, that the insoluble xylan of the stalk is directly related to the soluble pentosan in the grain and that one may be de-

rived from the other in the plant by addition or removal of arabinose side-chains. According to this scheme, xylan could be transported in the cell sap as the soluble arabinose-enriched pentosan and be deposited in this form, as in the grain, or as the insoluble arabinose-depleted xylan in other parts of the plant.

Acknowledgments

The author is indebted to Mr. J. Giroux for technical assistance and to Dr. G. A. Adams for continued interest. The kindness of Dr. F. Smith, Division of Agricultural Biochemistry, University of Minnesota, in supplying samples of 2,3-dimethyl-D-xylose and its anilide, is gratefully acknowledged. X-ray diffraction diagrams of the anilide were prepared by Dr. W. H. Barnes, National Research Laboratories, Ottawa.

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EFFECT OF STEAMING FRESH PADDY RICE ON THE DEVELOPMENT OF FREE FATTY ACIDS DURING STORAGE OF BROWN RICE [1,2]

D. F. HOUSTON, I. R. HUNTER, and E. B. KESTER

ABSTRACT

The rates of free fatty acid development at 25°C. (77°F.) have been determined in brown rice prepared from portions of freshly harvested moist paddy rice (21.9% H_2O, wet basis) which had been steamed various lengths of time at 88°, 93.5°, and 100°C. (190°, 200°, and 212°F.) before drying. The free fatty acids in the oil of the first storage series (13.4 to 13.9% H_2O), steamed 5 and 15 minutes at the three temperatures, increased by 2.1 to 2.9% during 140 days' storage compared with 10.0% for the unsteamed control (13.9% H_2O). The second series (12.9 to 13.4% H_2O), steamed 1, 2, 3, and 4 minutes at 100°C. (212°F.), increased by 1.7 to 2.1% in the same time and 5.7 to 5.8% in 285 days. The control (15.2% H_2O) showed an increase of 14.3% at 140 days and 13.8% at 285 days. Enzyme inactivation appeared essentially complete in all steamed samples.

The fatty acid development in the brown rice from steamed paddy followed an autocatalytic curve that can be expressed by $dF/dt = k(F)(100\text{-}F)$, where F is the percentage of free fatty acid in the oil, $(100\text{-}F)$ the percentage of unhydrolyzed oil, and t the time in days.

Additional effects of the steaming treatment are: (a) slight improvement in milling quality, (b) complete loss of viability, and (c) more rapid development of rancidity, as evidenced by peroxide numbers after 140 days' storage of 39.8 to 80.0 for the oil of samples in series No. 1, compared with 21.9 for the control.

Studies in this Laboratory (4) on rate of free fatty acid development in the oil of brown rice during storage have shown that it is markedly reduced by lowering the storage temperature or the moisture content of the grain. This is in accord with the general behavior of other grains and oil seeds in storage (3, 4, 11), and with the reported (2, 9) enzymatic nature of the process in rice. Inhibition of the enzyme can also be achieved by a short steam treatment of freshly harvested paddy rice (or of the separated bran), as a preliminary report (8) has shown. This paper records the effect of steaming the paddy on rate of development of free fatty acid during storage of subsequently prepared brown rice.

[1] Manuscript received February 19, 1951.

[2] Contribution from the Western Regional Research Laboratory, Albany, Calif. One of the laboratories of the Bureau of Agriculatural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Report of a study made under the Research and Marketing Act of 1946.

Materials and Methods

The single lot of Caloro rice from the 1948 harvest which was used had a moisture content of 21.9% as received. The rice was cleaned on a seed cleaner and a portion was used for steaming experiments on the day of receipt. The remainder was stored at 1.5°C. (35°F.) for seven weeks and then used in a second set of experiments. All moisture contents are given on a wet basis.

Steaming Treatment, Series No. 1. Five-pound samples of rice were treated with live steam or steam-air mixtures at 88°C. (190°F.), 93.5°C. (200°F.), and 100°C. (212°F.) for 5- and 15-minute periods on screen trays (2 ft. by 2 ft.) in a tunnel blancher. The rice layer was about 0.5 in. thick. Moisture contents after steaming (27 to 28%) were calculated from the weight of the rice upon emergence from the

TABLE I

PROCESSING DATA AND SAMPLE CHARACTERISTICS

Steaming Series No. 1

Steaming temp. (°F.)	Control	190		200		212	
Steaming time (min.)	0	5	15	5	15	5	15
% H_2O (wet basis)	13.9	13.6	13.7	13.6	13.9	13.5	13.4
Peroxide number[1] of oil after 140 days	21.9	39.8	66.2	57.4	76.0	80.0	76.8

Steaming Series No. 2

Steaming temp. (°F.)	Control	212			
Steaming time (min.)	0	1	2	3	4
% H_2O (wet basis)	15.2	12.9	13.2	13.1	13.4

[1] Millimoles of peroxide per 1,000 g. of oil.

blancher. The trays were placed at once in the air stream of a cabinet dryer at 43.5°C. (110°F.) and left until the weight loss indicated that the rice had dried to about 20% moisture. The samples were then placed in closed containers at room temperature overnight for moisture readjustment. This was followed by a second stage of drying at 43.5°C. (110°F.) to bring the moisture content to about 14% (Table I). A control sample was similarly dried (18 and 14%) without steam treatment. The dried rice was stored several days for moisture readjustment before milling.

Series No. 2. This series differed from the first only in that 10-lb. samples (1-in. layer of rice) were steamed at 100°C. (212°F.) for 1, 2, 3, and 4 minutes, and drying was performed in a single stage.

Milling and Storage. Brown rice for storage tests was prepared from the dried paddy by dehulling in laboratory-scale shelling stones

and cleaning in a seed cleaner. The resulting product consisted of whole grains plus a few large broken grains. Each sample was stored in a friction-top can at 25°C. (77°F.) for study of free fatty acid formation. Portions were removed at intervals for analysis. No attempt was made in these experiments to obtain sterile conditions.

Analysis. Determination of percentages of free fatty acid in the oil of the stored rice was performed by the modification of the Ames and Licata titration previously described (4). Peroxide numbers were determined according to Wheeler (10). Moisture determinations were made (1) by heating for 5 hours in a vacuum oven at 100°C. (212°F.).

Results and Discussion

That the steaming treatments in the first series were all excessive soon became evident from the storage tests, for the percentages of fatty acid developed were practically identical. Storage of this set of samples was continued, however, for 140 days to determine the general course of acid development in rice when the lipase was thoroughly inactivated.

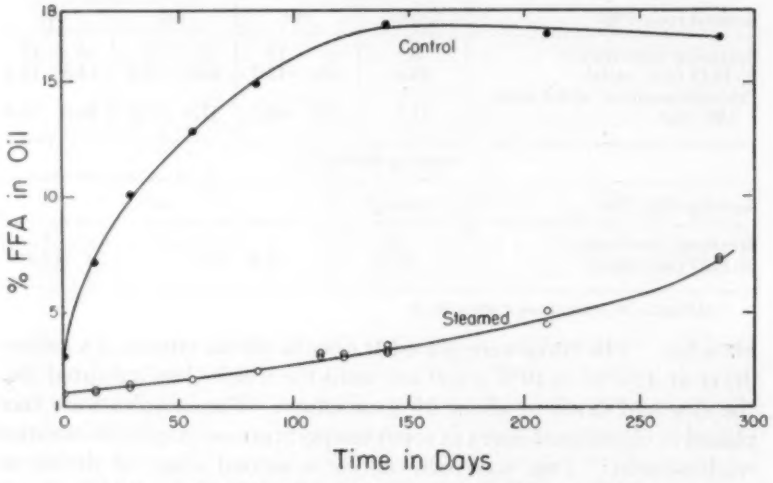

FIG. 1. Rate of free fatty acid formation in brown rice prepared from paddy steamed one, two, three, and four minutes at 212°F.

The percentages of free fatty acid developed during storage in the oil of the samples of brown rice from the second steaming series are presented in Fig. 1. The values for acid development are very similar, even at these short steaming times, and are of the same magnitude as those resulting from more rigorous treatment. Hence, it may fairly be assumed that as short a steam treatment as one minute at 100°C. (212°F.) destroys most of the lipolytic enzyme activity. This is cor-

roborated by the values for lipase activity previously reported (8) on the brans milled from these rices. Moisture contents of brown rice samples in both series are given in Table I.

Slight residual hydrolysis of fats during storage is evident in all steamed samples. The course of this action is, however, markedly different from that occurring in unsteamed controls, as is evident in Fig. 1. The difference between the two forms of curves is of the same nature as that found by Kyame and Altschul (6) in a study of immature and mature cottonseed. The development of fatty acid in untreated brown rice followed a course similar to that found in immature cottonseed, where the increase was attributed to enzymes which had not become dormant. The behavior of mature cottonseed, in which lipase activity is absent (7), was like that of steamed rice in which the enzymes have been inactivated.

The progression of lipolysis in the steamed rices can be expressed by the autocatalytic-type equation used by Karon and Altschul (5):

$$dF/dt = k\,(F)\,(100\text{-}F),$$

in which F = the percentage of free fatty acid formed, $100\text{-}F$ = percentage of residual unhydrolyzed fat, and t = number of days of storage. Integration and conversion to common logarithms yields the equation

$$\log F/(100\text{-}F) = 100kt/2.3 + \log F_0/(100\text{-}F_0),$$

in which F_0 is the original percentage of free fatty acid in the oil.

A plot of $\log F/(100\text{-}F)$ against t should give a straight line for an autocatalytic process. When the averaged percentages of free fatty acid for each series of steamed rices are plotted aginst storage times, the resulting points fall on practically straight lines, as is shown in Fig. 2. The numerical values of the rate constant, k, evaluated from the slopes of the lines, are 7 and 6×10^{-5} reciprocal days for Series No. 1 (13.6% H_2O) and No. 2 (13.1% H_2O), respectively. The magnitude of these constants is in agreement with the values found by Karon and Altschul (5) for mature cottonseed, and shows an increase with moisture content as would be expected.

Additional effects of steaming the paddy are also evident. Complete loss of germinating power is found after as little as one minute steaming at 100°C. (212°F.). The milling quality of the rice appears somewhat improved. This is in keeping with the relation of the treatment to those used in making parboiled rice, which shows a much higher milling yield than untreated rice. Further investigation has been made on the milling of steamed rice and will be reported separately. Partial loss in resistance to oxidation results from steaming the rough

rice. Peroxide values shown in Table I indicate that, after 140 days' storage, oxidation has proceeded further in the steamed rice than in the control, and is greater with more severe steaming treatment. In addition, the steamed samples from Series No. 2 after 285 days' storage all had a rancid odor which was not evident in the control.

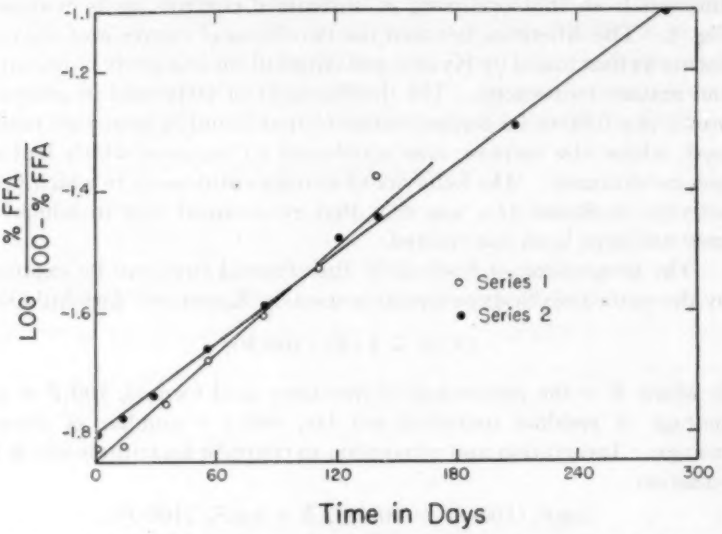

FIG. 2. The relation between storage time and a function of the percentage of free fatty acid formed in the oil of brown rice prepared from steamed paddy. Series No. 1. Steamed 5 and 15 minutes at 190°F., 200°F., and 212°F. Series No. 2. Steamed 1, 2, 3, and 4 minutes at 212°F.

The value of the steaming treatment in improving milling quality and reducing rate of development of free fatty acid is somewhat offset by the loss in resistance to oxidation and in viability, which would make the rice more susceptible to growth of micro-organisms. As low fatty acid content is most important in recovery of oil from rice bran, and oxidation resistance is of relatively greater value in storing brown rice, the present results may be interpreted as showing that the previously reported steaming of bran (8) has greater practical usefulness than steaming of field-wet paddy rice.

Acknowledgment

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PREPARATION OF RIBONUCLEOPROTEIN AND RIBONUCLEIC ACID FROM WHEAT GERM [1]

C. V. LUSENA [2]

ABSTRACT

Ribonucleoprotein was prepared by extracting fresh wheat germ meal with water for 10 minutes at room temperature, centrifuging, adjusting the pH of the supernatant extract to 4.0 and centrifuging. The nucleoprotein precipitate was purified by redissolving at pH 6.5–7.0 and reprecipitating at pH 4.0 twice. Yields of ribonucleic acid bound with protein were 2.2% of the wheat germ. Nucleoprotein solutions at pH 11 were heated for three hours at 80°C. to break the nucleic acid-protein bond. Adding ethanol to a concentration of 40% and adjusting the pH to 7 precipitated most of the protein. After the supernatant solution was concentrated to 2% nucleic acid and the pH was adjusted to 4.0, 1.1% of the wheat germ was recovered as nucleic acid, 95% pure. This preparation was redissolved at pH 6 and reprecipitated with ethanol at pH 4.0 twice; the yield was then 0.8% of 99.6% pure ribonucleic acid.

Ribonucleic acid (RNA) preparations from wheat germ and from yeast have given similar hydrolytic products and it has been suggested that they are similar in constitution (4, 10, 16, 21). RNA constitutes about 3.6% of wheat germ (19); nevertheless yields of over 1% have not been reported and examination of the phosphorus analyses suggests that the best preparations were only 80% pure.

Most investigators have used the method of Osborne and Harris (20), which consists of precipitating a nucleoprotein from an aqueous extract of wheat germ meal by saturating it with sodium chloride; then, after vigorous peptic digestion, the residue is washed and dissolved in potassium hydroxide, and precipitated with hydrochloric acid three times. Clarke and Schryver (7) on the other hand, released the RNA from the protein by boiling wheat germ meal in ethyl alcohol and then extracting it directly with 10% sodium chloride; both yield and purity were low.

This investigation was designed to improve the purity of RNA obtained from wheat germ, through a preliminary separation of the nucleoprotein.

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Contribution from the Department of Chemistry, Faculty of Agriculture (McGill University) Macdonald College, Quebec. This paper constitutes part of a thesis submitted to McGill University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.
[2] Research Assistant. Present address: Division of Applied Biology, National Research Laboratories, Ottawa.

Material and Methods

Fresh commercial wheat germ containing less than 5% impurities was finely ground and stored at −10°C. in closed containers.

Nitrogen was determined by a micro-Kjeldahl method (6), pH with a glass electrode (3), phosphorus and sodium gravimetrically (1, 2), moisture at 70°C. under vacuum and RNA colorimetrically with orcinol (17).

Preparation of Nucleoproteins. Preliminary experiments showed that the protein of wheat germ was most effectively extracted by shaking the germ (1 part) with water (10–15 parts) for 10 minutes at room temperature. Fresh wheat germ was found to give more protein than germ that had been kept for two months at 5°C. About 61% of the total nitrogen was extracted from one part of fresh wheat germ with 10 parts of water in 10 minutes shaking.

Ten milliliter portions of dilute hydrochloric acid of various concentrations were mixed with 20 ml. aliquots of wheat germ extract containing 2.2 mg. of nitrogen per ml. Each was allowed to stand one hour and then centrifuged at 2,000 times gravity for 10 minutes. The supernatant solutions were analyzed for nitrogen and their pH determined. The results (Fig. 1, curve A) show that maximum precipitation of the protein (i.e., 68% nitrogen) occurred at pH 4. Since about 19% of the extracted nitrogen is non-protein nitrogen, therefore the protein nitrogen, which remained in solution, represented only 13% of the original nitrogen extracted.

The protein precipitated at pH 4.0 was redissolved at pH 6.5 and reprecipitated at pH 4.0 twice, then dissolved and reprecipitated at different pH values. The results (Fig. 1, curve B) show that repeated precipitation gave a greater recovery of protein in the precipitate and that the pH for maximum precipitation was 4.0.

After three precipitations at pH 4.0, the material had a high content of phosphorus and pentose, corresponding to about 21% RNA, or about 2.2% of the wheat germ expressed as RNA. Solutions of this precipitate were prepared at pH 6.5, adjusted to different pH levels with hydrochloric acid, and centrifuged; the supernatant solutions were analyzed for nitrogen and pentose. The results (Table I) show that pH 4.0 is best for maximum precipitation of RNA from nucleoprotein solutions. The proportions of RNA in the dry matter of the supernatant solutions, calculated from the nitrogen: pentose ratio, indicate variability in the composition of the precipitate at various pH levels. At pH 4.0 the nucleoprotein precipitated had a higher content of RNA than the solution from which it was derived. Since RNA split from the protein was more soluble at pH 4.0 for maximum recovery

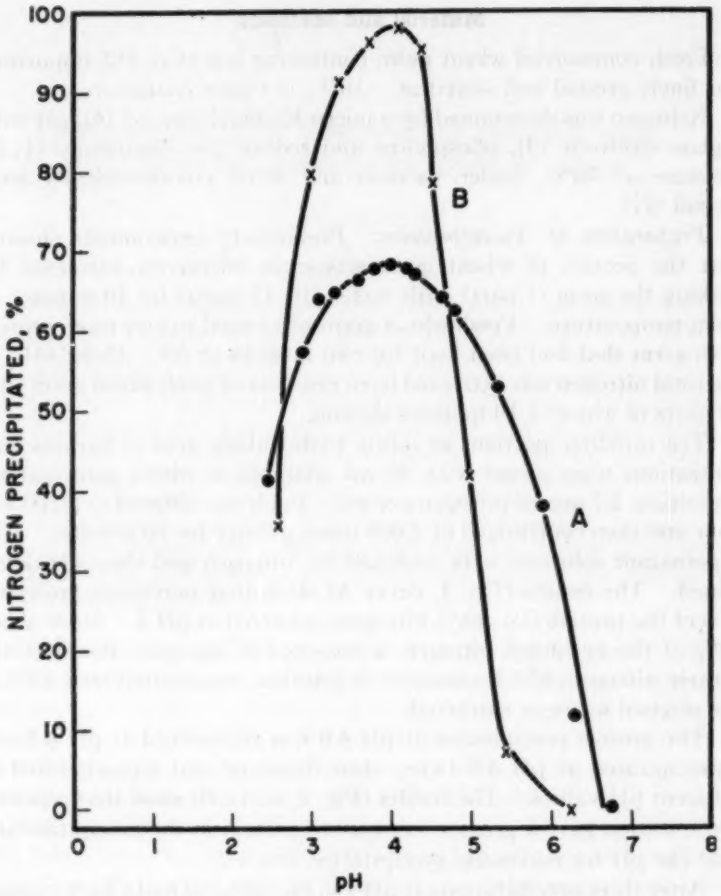

FIG. 1. The effect of pH on the solubility of ribonucleoprotein.

of RNA, extraction and reprecipitation should be carried out under as mild conditions as possible to avoid dissociation of RNA and protein.

Preparation of Crude RNA. In preliminary trials to separate RNA from the protein it was found necessary to adjust the nucleoprotein solution to pH 11, heating for three hours at 80°C. (milder conditions were insufficient to disrupt the association, more drastic conditions rendered the protein too soluble). After this treatment the solubility of the RNA was greatly increased but the pH for minimum solubility was still 4. The amount of protein precipitated was almost as great as for fresh preparation, but the pH for minimum solubility was shifted to a higher value of about pH 4.65.

TABLE I

EFFECT OF pH ON SOLUBILITY OF A THRICE-PRECIPITATED NUCLEOPROTEIN

pH	Nitrogen Precipitated	Pentose Precipitated	RNA in Solution, Dry Matter
	%	%	%
5.95	2.1	1.2	19.8
5.65	5.4	1.8	20.3
5.40	8.5	4.8	20.4
5.20	11.3	5.0	21.1
5.00	45.2	34.6	21.5
4.90	76.9	73.6	22.3
4.45	92.9	91.2	24.7
4.00	94.5	98.1	18.9
3.70	92.0	94.4	15.0
3.00	50.5	61.9	15.2
2.60	37.4	48.7	16.0

The two pH levels for maximum precipitation (4.0 and 4.65) were too close to give a sharp separation. Normally, the addition of ethyl alcohol shifts the titration curve of proteins towards a higher pH. Adding the alcohol either before or after the heating period to a concentration of 40% was found most suitable: higher concentrations increased the losses of RNA in the protein precipitate, and lower concentrations did not markedly separate the pH of protein precipitation from the pH for RNA precipitation.

TABLE II

THE EFFECT OF pH ON THE PRECIPITATION OF A DENATURED NUCLEOPROTEIN IN 40% ETHANOL

	In Supernatant Solution		
pH	Nitrogen	Pentose	Purity of RNA
	%	%	%
8.6	43.3	99.0	41.8
7.8	39.6	100.1	47.6
7.3	36.5	100.1	51.5
6.6	30.2	96.3	58.3
5.7	25.5	89.7	64.9
4.8	22.2	72.2	59.9
4.1	20.9	56.6	49.6

A solution of purified nucleoprotein at pH 11 was heated for three hours at 80°C. Alcohol was then added to 40% concentration and the pH was adjusted to various levels ranging from 4.1 to 8.6. Table II shows that the pH for maximum precipitation of the protein without RNA is then between 6.6 and 7.3. When the local concentration of acid was reduced to a minimum by adding dilute acid through a fine bore pipette immersed in a large volume of vigorously stirred solution,

complete recovery of RNA in the supernatant solution was obtained at pH 7.0 with a purity of 62%. The recovery of crude RNA in solution varied from 85% to 97% with the purity ranging from 74%–90%.

Purification of RNA. The crude RNA solutions were concentrated under vacuum at 50°–60°C. to at least 2% RNA, centrifuged to remove a protein sludge, and the pH adjusted to 4.0. The precipitate was a nucleoprotein containing 25%–50% RNA. RNA was recovered from the clear supernatant solution by adding ethanol to 60% concentration. The light white powder recovered after drying with ethanol and ether represented about 1.1% of the wheat germ and was better than 95% pure RNA. These preparations were purified as follows:

A 1.5 g. portion of a RNA preparation, equivalent to about 1.2 g. of pure RNA (allowing for moisture, sodium, and impurities), was suspended in 40 ml. of 0.025 *N* sodium hydroxide. The resulting turbid solution at pH 5.7 was brought to pH 4.0 and centrifuged after standing one hour. To the clear supernatant solution 60 ml. of absolute ethyl alcohol was added and, after two hours, the precipitate was recovered by centrifuging. The RNA was then dissolved in 25 ml. of 0.02 *N* sodium hydroxide. When the pH of this solution was adjusted to 4.0, very little precipitate was obtained. Forty milliliters of absolute alcohol was added to the clear supernatant solution. After two hours, the precipitate was collected by centrifuging, washed with ethanol and ethyl ether, and dried under vacuum at 50°C. The white powder (0.9 g.) recovered corresponded to about 70% of the first crude RNA precipitated. Analysis showed that it was 99% pure after correction for its sodium and water content.

TABLE III

ANALYSES OF SOME RNA PREPARATIONS

RNA Sample	1	2	3	Schwarz	Theoretica
Moisture, %	7.9	8.5	2.5	8.7	0
Sodium, %	2.8	3.2	2.6	1.0	0
Nitrogen, % (dry and Na-free)	15.33	15.52	16.01	15.96	16.12
Phosphorus, % (dry and Na-free)	8.91	9.08	9.49	9.40	9.52
N:P ratio	1.72	1.71	1.69	1.70	1.69
Purity, % based on nitrogen	95.1	96.3	99.3	99.0	100.0
Purity, % based on phosphorus	93.6	96.4	99.7	98.7	100.0
Reacting pentose, % (dry and Na-free)	21.74	22.07	22.96	22.86	23.01[1]
N:pentose ratio	0.705	0.703	0.697	0.698	0.70

[1] One-half theoretical content, because only the ribose linked to purine bases reacts with orcinol (17).

Some typical analytical results of RNA preparations are shown in Table III. The theoretical values were calculated on the assumption

that the basic unit for wheat germ RNA is the "statistical" tetranucleotide structure postulated by Levene (15) for yeast RNA. For comparison, analyses were carreid out on yeast RNA prepared by Schwarz Laboratories Inc. (lot HN 4531), 97%–99% pure. RNA-1 was prepared from 1.8 kg. of wheat germ with a yield of 1.2%. RNA-2 was prepared from 1 kg. of wheat germ with a yield of 1.1%. RNA-3

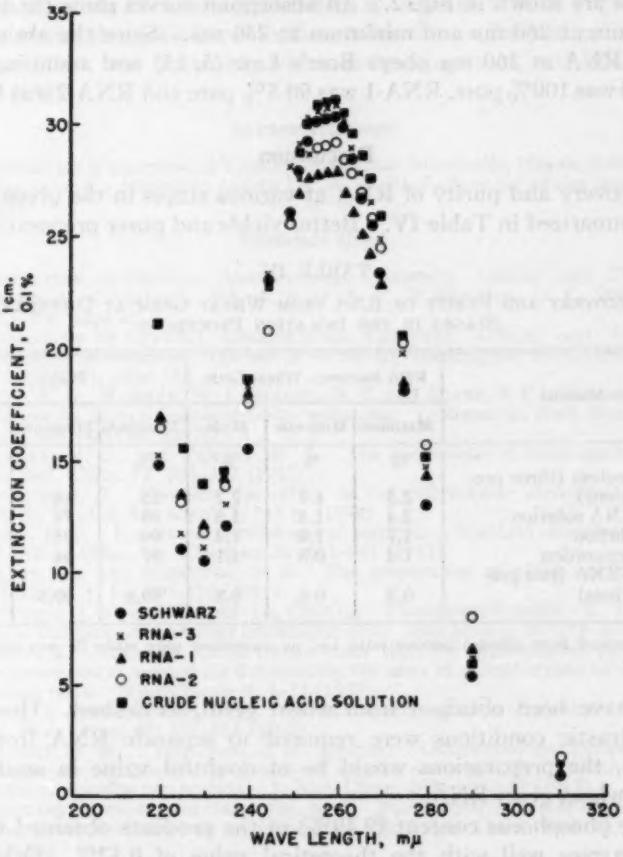

FIG. 2. Light absorption spectra of ribonucleic acid preparations.

was obtained by reprecipitating a portion of RNA-1 twice, with a recovery of 75% (i.e., it represents about 0.75% of the wheat germ). Phosphorus and nitrogen determinations on the RNA-3 and Schwarz samples gave values agreeing closely with the theoretical. All RNA preparations gave a negative test with Dische diphenylamine reagent (9), indicating the absence of desoxyribonucleic acid. Solutions were

prepared of the same samples and also of crude RNA containing 20% protein impurities, which were taken into account in calculating the concentration, in 0.005 *N* phosphate buffer at pH 7 and in 0.001 *N* sodium hydroxide. (The actual pH of the solutions varied between 7.12 and 7.15). The calculated extinction coefficients for a 1 cm. layer of 0.1% solution (1 mg. per ml.) of the moisture- and sodium-free samples are shown in Fig. 2. All absorption curves show the typical maximum at 260 mμ and minimum at 230 mμ. Since the absorption of the RNA at 260 mμ obeys Beer's Law (5, 13) and assuming that RNA-3 was 100% pure, RNA-1 was 90.5% pure and RNA-2 was 94.5% pure.

Discussion

Recovery and purity of RNA at various stages in the preparation are summarized in Table IV. Better yields and purer preparations of

TABLE IV

RECOVERY AND PURITY OF RNA FROM WHEAT GERM AT DIFFERENT STAGES IN THE ISOLATION PROCEDURE

Material	RNA Recovery, Wheat Germ			Purity		
	Maximum	Minimum	Mode	Maximum	Minimum	Mode
	%	%	%	%	%	%
Nucleoprotein (three precipitations)	2.5	1.7	2.2	25	16	21[1]
Crude RNA solution	2.4	1.4	1.8	90	74	80[1]
RNA solution	1.7	1.0	1.2	99	95	97[1]
RNA preparation	1.4	0.8	1.1	97	94	95
Purified RNA (two precipitations)	0.9	0.6	0.8	99.8	99.5	99.6

[1] Calculated from nitrogen:pentose ratio, i.e., no corrections were made for non-nitrogenous impurities.

RNA have been obtained from wheat germ, as desired. However, since drastic conditions were required to separate RNA from the protein, the preparations would be of doubtful value in studies of native wheat germ RNA.

The phosphorus content (9.49%) of the products obtained in this study agrees well with the theoretical value of 9.52%. Other investigators have explained values varying from 7.5% to 9% on the basis of loss of phosphoric acid (8). Khouvine and Gregoire (12) reported 9.3% phosphours for RNA from *Calliphera erythrocephala*, and Vendrely *et al.* reported 9.59% from yeast (22). The commercial preparation used for comparison contained 99.4% of the theoretical amount of phosphorus. Exactly 50% of the ribose in RNA reacted with orcinol as already described (17).

The ultraviolet absorption spectrum of purified RNA from wheat germ is identical with that of a very pure sample of yeast RNA, prepared by Schwarz Laboratories. However, the extinction coefficient at 260 mμ. is difficult to compare with other values reported in the literature (8, 14, 18), because the calculation of these values is not clearly expressed and the purity of the samples is not reported. When the values in Fig. 2 were recalculated, a value of 0.98×10^{-4} for an average molecular extinction coefficient for the purine and pyrimidine bases in RNA was obtained, which is in agreement with Kalckar's results (11).

Acknowledgments

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FUNGI ON AND IN WHEAT SEED [1,2]

CLYDE M. CHRISTENSEN [3]

ABSTRACT

Fungi known to cause deterioration of moist stored wheat were found as dormant spores on the surface of the seed, and as dormant mycelium within the pericarp. The inoculum occurring as external, dormant spores was much more abundant than that in the form of living, internal mycelium. Both external and internal inoculum were more abundant in the low-grade lots than in those of high quality.

Mycelium was present beneath the pericarp of all of the seeds examined. Most of this mycelium was dead. In the high-grade lots, most of the living mycelium beneath the pericarp was that of Alternaria, a fungus not known to cause deterioration of stored seeds. In the low-grade lots, most of the living mycelium beneath the pericarp was that of Aspergillus and Penicillium known to be involved in the deterioration of stored grain. No living mycelium was found in seeds more than eight years old.

It is well known that various fungi occur on and within the seed of wheat, and that some of these fungi cause deterioration of the stored grain (2, 4, 6, 7, 8). Carter (2) found spores of various fungi to be common on the outside of wheat seed; in general, the numbers of these spores increased as the quality of the seed decreased. Oxley and Jones (9) found mycelium to be common beneath the pericarp of wheat seed. Hyde (5) found mycelium beneath the pericarp of all but one of more than 260 samples of wheat seed collected from the major wheat growing regions of the world.

The evidence now available indicates that nearly all wheat seed is "moldy." Different lots of seed differ in the number and kinds of external and internal fungi present, but no samples of commercial wheat have been found to be free of molds.

Oxley and Jones (9) suggested that subepidermal mycelium might be a major source of inoculum of fungi that cause deterioration of

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stored wheat. They removed the pericarp from wheat seeds, and found that when such seed was moistened it respired at a relatively low rate. Removing the pericarp would remove most of the external spores as well as the mycelium within the pericarp. It might also remove that portion of the seed on which the fungi concerned could grow most vigorously. Their results gave no information as to the relative importance of dormant, external spores vs. dormant, internal mycelium, as a source of inoculum of those fungi that might invade the seed in storage. Hyde (5) did not determine whether the mycelium observed beneath the pericarp of wheat seed was living or dead, or to what fungi it belonged.

The work here reported was undertaken to determine: 1. The relative amount of external and internal inoculum of storage molds (essentially those fungi able to grow on grain whose moisture content is in equilibrium with relative humidities of from 65 to 90%). 2. The proportion of living mycelium beneath the pericarp, and the identity of this living mycelium.

Materials and Methods

Source of Seed. All but a few of the samples were from commercial lots of the 1948 crop. Geographically, their source ranged from North Dakota to Texas. The "high-grade" lots were mostly of the top commercial grade. The "low-grade" lots contained from 10 to 30% damaged seed. All of the samples were stored in a refrigerator at about 5°C. from the time they were received, late in 1948, until work was begun on them late in 1949. A few samples of seed from 5 to 60 years old, mostly from the midwestern United States, were included in the study.

Determination of External Inoculum. Ten grams of seed were selected from each lot.[4] The seeds were examined with a stereoscopic microscope, under a magnification of 10×, and only unbroken seeds with no visible cracks in the pericarp and no hole at the attachment end were chosen.

The seeds so selected were placed in a 0.1% solution of agar (this was found preferable to water alone, as it prevented the settling out of spores) shaken on a mechanical shaker at 400 strokes per minute for one-half hour, diluted in a series of 0.1% agar solutions, and cultured in malt-salt agar, known to be favorable to the cultivation of storage molds (3).

Internal Inoculum. Seeds were selected as described above. This careful selection was necessary because preliminary work had shown that

[4] This rather painstaking selection was necessary to determine the amount and kind of living mycelium within the pericarp, and so an equally rigorous selection was used to determine external inoculum.

if seeds with cracked pericarps, or with holes near the point of attachment were included, fungus spores were likely to be present under the pericarp, and they could not be removed by washing. Thirty to 40 selected seeds of each lot were washed under running tap water at 35° to 40°C. for several hours, a small amount of detergent being added at intervals of about one-half hour. The seeds were then rinsed in several changes of sterile distilled water, and individual seeds were taken with sterile tweezers, the brush end cut off with a sterile razor blade.[5] A strip of pericarp 1 to 2 mm. wide, extending from the germ end to just below the brush, was removed and placed inner-side-up on a thin film of agar on a cover slip. The cover slip was placed on a van Tiegham cell in a Petri dish. Van Tiegham cell cultures are relatively free from chance contamination by air-borne spores, and in the present work, many of the cultures kept for weeks without contamination.

The strips so cultured were examined microscopically under low power (×100) at intervals of 12 to 24 hours for several days. If a hypha grew from mycelium within the pericarp, it was examined with high power (×400) to determine its place and mode of origin. All of the cultures listed later in the paper as arising from dormant mycelium within the pericarp were actually observed to arise from cells of dormant mycelium. Mycelium on the inner side of the pericarp, or between the layers of the pericarp, could be observed in these strips. By observing the hyphae that developed from this mycelium, it was possible to determine how much of the mycelium was alive. The identity of this living mycelium could be determined when the fungus (or fungi, if several of them grew from the same strip) produced spores. If the fungus did not sporulate within a few days, as was often the case with Alternaria, it was transferred to an agar slant in a test tube, and identified later, after it had sporulated.

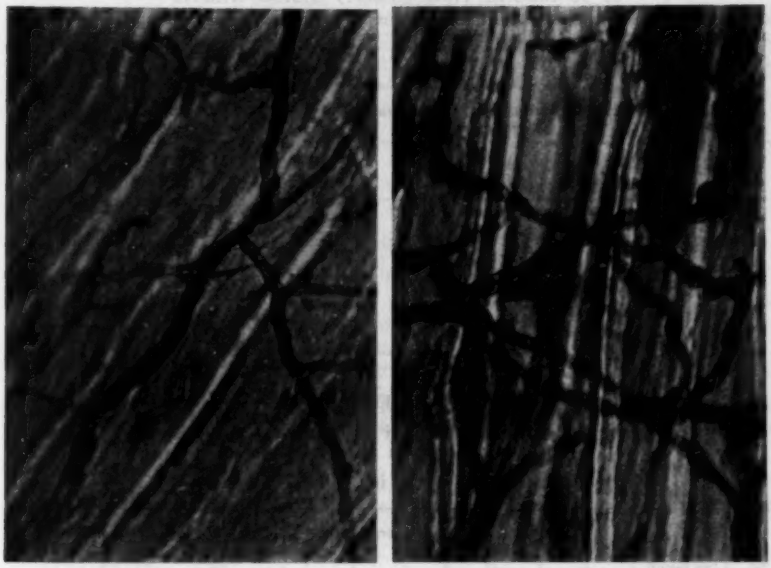
One strip of pericarp was cultured from each of 20 seeds of each of ten high-grade lots, and from each of seven low-grade lots. The position of the strip varied from near the crease to the middle of the rounded side of the seed. Each strip included a portion over the embryo, where in the experience of Hyde (5), as well as that of the writer, mycelium is most likely to be present.

Results

Mycelium was present between the cell layers of the pericarp, or on the inner side of the pericarp, or both, in all of the strips cultured, from both high-grade and low-grade lots. Typical specimens are

[5] The brush end was cut off because it was found that spores, mycelium, and sclerotia sometimes adhered so tenaciously to the hairs of the brush that they could not be washed off, and germinated so quickly that they obscured cultures arising from the inner pericarp.

illustrated in Fig. 1. In all cases, most of this mycelium was dead. In the high-grade lots, most of the living mycelium was that of Alternaria, a fungus of no known significance in the deterioration of stored seed (1, 8, 10). In the low-grade lots, the majority of fungus colonies from dormant mycelium within the pericarp were of fungi known to cause deterioration in stored seed.



A. B.

FIG. 1. Fungus mycelium within the pericarp of wheat seed. A. Mycelium principally on the inner side of the pericarp. B. Mycelium principally between the layers of the pericarp.

Mycelium was abundant within the pericarp of seeds from five to 60 years old, but almost all of it was dead. A single colony of Alternaria was obtained from dormant mycelium within the pericarp strips from 20 seeds of a lot eight years old. No storage molds were obtained from dormant internal mycelium in seeds more than five years old, and no fungi at all grew from mycelium within the pericarp of seeds more than eight years old. From 50 to 75% of the seeds up to 15 years old were viable, and a few seeds of a lot of Marquis 27 years old germinated, indicating that the seeds retained their viability longer than did the dormant mycelium under the pericarp.

An average of 18 colonies of various storage molds were obtained from the outside of each seed of the high quality lots. Only two colonies of storage molds grew from the pericarp strips of 200 seeds in the high-quality lots. Assuming that only one-tenth of the pericarp

on each seed was cultured, and that the strips cultured were a fair sample, there were only 20 colonies of storage molds within the pericarp of 200 seeds, or one colony for every ten seeds. These data are summarized in Tables I and II.

TABLE I

FUNGI CULTURED FROM INNER PERICARPS OF 20 SEEDS FROM EACH OF 10 LOTS OF HIGH QUALITY SPRING WHEATS

Seed Lot	No. from Which Fungi Were Cultured	Fungi Significant in Storage		Fungi not Significant in Storage		
		Aspergillus glaucus	*Penicillium* spp.	*Alternaria*	*Helmintho-sporium*	Other
1	6	0	1	4	1	0
2	7	0	0	7	0	0
3	0	0	0	0	0	0
4	10	0	0	7	3	0
5	6	0	0	4	2	0
6	11	1	0	8	0	2
7	6	0	0	6	0	0
8	3	0	0	2	1	0
9	3	0	0	3	0	0
10	13	0	0	13	0	0

TABLE II

NUMBERS OF COLONIES OF FUNGI FROM THE OUTSIDE OF 10 LOTS OF HIGH QUALITY WHEATS

(Same lots as in Table I)

Lot No.	Numbers Colonies per Gram of Seed	Fungi Significant in Storage				Fungi not Significant in Storage		
		Aspergillus glaucus	*Aspergillus candidus*	*Penicillium* spp.	Others	*Alternaria*	*Helmintho-sporium*	*Fusarium*
1	220	70	5	5	0	0	20	0
2	280	70	20	5	0	0	5	0
3	240	70	10	0	0	20	0	0
4	960	25	0	2	2	30	35	16
5	420	60	10	10	10	0	10	0
6	2,220	70	0	25	5	0	0	0
7	520	30	0	20	0	40	10	0
8	620	75	0	20	0	2	3	0
9	40	50	0	0	0	0	0	50
10	600	20	0	40	10	0	0	30
Mean 612 Colonies/gram			453				159	
Colonies/seed			18				6	

In the low-quality lots, an average of 3,000 colonies of storage molds were obtained from the outside of each seed, while only three colonies of storage molds grew from dormant mycelium within the pericarp of each seed (Tables III and IV). It was not unusual for

TABLE III

FUNGI CULTURED FORM THE INNER PERICARP OF 20 SEEDS FROM EACH OF SEVEN LOTS OF LOW-GRADE WHEAT

Lot No.	Total Yielding Fungi	Significant in Storage				Not Significant in Storage
		Aspergillus glaucus	*Aspergillus tamarii*	*Aspergillus flavus*	Others	*Alternaria*
11	19	5	14	8	2	1
12	17	11	7	0	2	0
13	14	4	0	0	3	9
14	9	8	0	0	0	1
15	12	7	0	0	1	7
16	10	0	4	1	0	5
17	13	6	4	0	0	5

TABLE IV

NUMBERS OF COLONIES OF FUNGI FROM THE OUTSIDE OF SEVEN LOTS OF LOW-QUALITY WHEATS

Lot No.	Colonies per Gram	Fungi Present in Per Cent of Total (All Storage Fungi)						
		Aspergillus glaucus	*Aspergillus versicolor*	*Aspergillus niger*	*Aspergillus flavus*	*Aspergillus ochraceus*	*Penicillium*	Other
11	72,000	76	7	3	4	3	4	4
12	103,000	74	1	15	9	1	10	0
13	46,000	91	0	4	0	0	5	0
14	230,000	78	0	1	7	0	2	12
15	1,200	100	0	0	0	0	0	0
16	34,000	89	0	0	0	0	11	0
17	83,000	80	4	4	0	0	8	4
Broken or Otherwise Damaged Seeds of the Above Yielded								
11	268,000	68	7	7	3	3	8	4
12	212,000	95	0	0	0	0	5	0

several different species of Aspergillus and one or more colonies of Penicillium to grow from dormant mycelium within a single strip of pericarp from seeds in the low-grade lots.

A single fungus colony originating from dormant mycelium within or beneath the pericarp sometimes arose as one or a few hyphae, as shown in Fig. 2. Sometimes the colony arose as hundreds of hyphae growing from a multitude of living cells throughout the inner side of the pericarp. Sometimes (almost only in low-grade lots) it arose as dozens of hyphae growing from microsclerotia that filled one or more cells of the inner layer of the pericarp, as shown in Fig. 3. Where many hyphae arose simultaneously from internal mycelium, they were

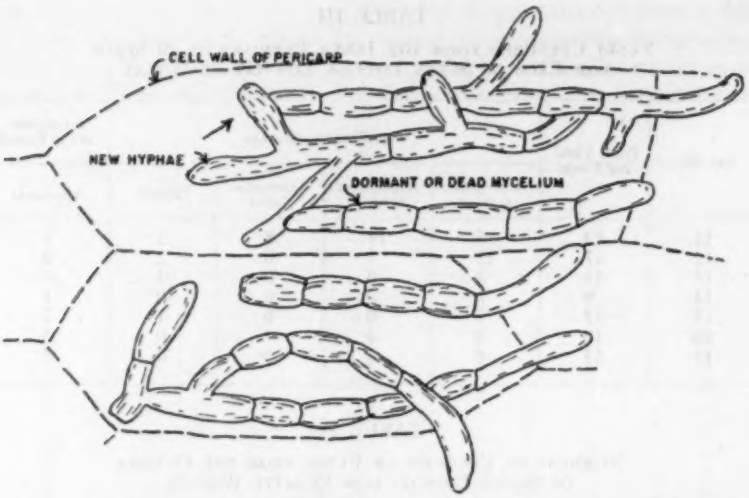


FIG. 2. Germinating mycelium of Alternaria within 2 cells of the inner pericarp of wheat seed. Mycelium was generally present under the pericarp, but all of it except that in these 2 host cells was dead. Drawn 20 hours after the pericarp strip was cultured.

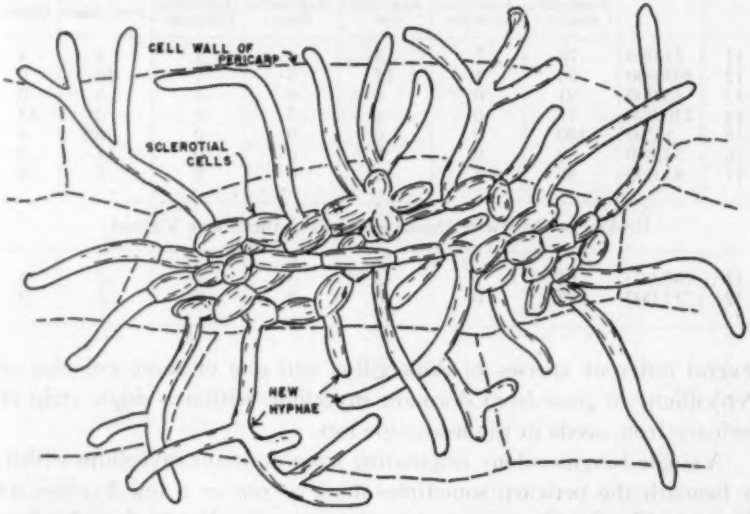


FIG. 3. Germinating sclerotial cells of *Aspergillus tamarii* in the inner pericarp of wheat seed. In this strip of pericarp, only a single host cell contained living mycelium, although dead mycelium was present throughout the inner portion of the strip. Drawn 20 hours after the strip was cultured.

counted as a single colony. If the seed was stored under conditions where these fungi could grow, such internal inoculum presumably would be equivalent to a rather heavy external spore load.

Discussion

Many varieties of fungi were present on and within the wheat seed studied. Relatively little of the mycelium within the pericarp was alive. In the high-grade lots little of the living mycelium was that of fungi known to cause deterioration of the stored seed. However, some dormant mycelium of storage molds was present internally in even the best seeds of high-grade wheats. If it were practically possible to eliminate the external spores and mycelium, even high-quality seed would by no means be free of storage molds. Living internal mycelium of storage molds was considerably more abundant in the best seeds of low-quality lots than in those of high-quality.

External inoculum of storage molds was more abundant than internal inoculum in all of the lots studied, but the relative importance of external vs. internal inoculum is difficult to evaluate. Under the conditions encountered in practice, external and internal inoculum both probably are involved in the deterioration of grain in storage. The relative importance of each probably varies with such factors as the amount present, the conditions of storage, and the age of the seed.

The present studies were limited to an investigation of the fungus inoculum on the surface and immediately beneath the pericarp of wheat seed. Eventually it must be ascertained how and under what conditions these fungi invade the germ and endosperm of the seed; it is not only necessary to measure their numbers and kinds, but also their effects on the major physical and biochemical properties of the seed.

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THE CHROMATOGRAPHIC EXAMINATION OF SUGARS IN WHEAT FLOUR [1,2]

KENNETH T. WILLIAMS and ARTHUR BEVENUE

ABSTRACT

Wheat flour carbohydrates soluble in 80% ethyl alcohol were examined by paper chromatographic techniques. Four commercially-milled flours of different types gave chromatograms that indicated that the same sugars were present in each of the flours. Fructose, glucose, sucrose, maltose, raffinose, and arabinose were identified. In addition a slow-moving spot, assumed to be Tanret's levosine, was resolved by extended periods of irrigation into at least seven substances, each possessing a ketose structure and containing fructofuranoside linkage. All, except the material remaining at the starting line of the chromatograms, are fermentable by bakers' yeast. These seven fractions represented about 75% of the reducing power of the alcohol-soluble carbohydrates.

Investigations at this laboratory of factors affecting the baking quality of wheat flour (15, 16) have necessitated more detailed knowledge of the substances present in flour. For example, the identity of the sugars is still questionable. The reviews by Atkin *et al.* (5) and Bailey (6) of the literature on sugars in wheat flour illustrates the lack of definitive identification of the sugars present. They are usually described as reducing sugars, non-reducing sugars, hexoses, sucrose, sugars as maltose, and levosine.

Levosine was the name given by Tanret (18) to a carbohydrate fraction isolated from flour, and for which he proposed the formula $(C_{12}H_{10}O_{10})_4$. Bailey's review (6) of the literature on levosine includes the facts that hydrolysis yields 9 parts fructose to 1 part of a hexose considered to be glucose; that it is not hydrolyzed by invertase; that white flour contains 0.4 to 1.5% with an average of 0.7% of this substance; and that conflicting views exist concerning its fermentability by bakers' yeast. Although this substance represents more than one-half of the material measured as sugar in flour, no investigational reports on it have been found in recent literature. It seemed possible that the "purified" levosine was still quite heterogeneous. Therefore, a study of the carbohydrate fractions soluble in 80% ethyl alcohol was made using paper chromatographic techniques. The results obtained on four flours of different types are given in this report.

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[2] Contribution from the Western Regional Research Laboratory, Albany, California. One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

Materials and Methods

Four commercially-milled wheat flours were used in this investigation. Their description and analytical data are given in Table I. The moisture, ash, and nitrogen contents of the flours were determined by the methods described in Cereal Laboratory Methods (1).

TABLE I

DATA ON THE WHEAT FLOURS USED FOR CHROMATOGRAPHIC STUDIES [1]

Type of Flour	Protein	Ash	Reducing[2] Sugars	Total Sugars[2]	
				Invertase Hydrolysis	HCl Hydrolysis
	%	%	%	%	%
"All-purpose" family	10.29	0.33	0.11	1.10	1.22
Bakers Patent, bleached, No. 1	12.27	0.41	0.06	0.77	0.87
Bakers Patent, bleached, No. 2	11.51	0.37	0.09	1.09	1.27
Cracker, unbleached	10.57	0.39	0.08	1.02	1.15

[1] All analytical data converted to 14% moisture basis.
[2] As glucose.

Preparation of Flour Extracts. An 80% alcoholic extract of each flour sample was prepared and diluted to a definite volume (21). A portion of each of the alcoholic extracts was evaporated on a steam both to remove the alcohol, cooled, filtered through a mat of Celite Analytical Filter-Aid[3], and made to a definite volume with distilled water. Aliquots of this solution were used without any additional clarification (7). Additional aliquots of this aqueous solution were clarified further by the neutral lead acetate procedure (3), and other aliquots were clarified by treatment with ion-exchange resins as previously described (20). (The word clarification, as used throughout this report, refers to the removal of non-sugar reducing substances from the flour extracts.) Portions of these solutions were analyzed for reducing and non-reducing sugars (2, 4). Other portions of these solutions were concentrated by evaporation on a steam bath, a crystal of thymol was added, and the concentrates were stored in the refrigerator until required for paper chromatographic studies. The three types of clarified solutions gave the same carbohydrate pattern on the paper chromatogram. In the greater part of this investigation, ion-exchange clarification was used.

Paper Chromatography. The paper chromatographic technique was essentially that used by Partridge (12, 13). All of the chromatograms were made on Whatman No. 1 filter paper. The solvent mixtures

[3] Mention of trade names of material does not constitute endorsement by the Department of Agriculture.

used for irrigating the chromatograms were 1-butanol-ethanol-water (10:1:2), benzyl alcohol-glacial acetic acid-water (3:1:3), ethyl acetate-pyridine-water (8:2:1), water-saturated phenol, and water-saturated collidine (10, 13). Of all the mixtures investigated the 1-butanol-ethanol-water and the ethyl acetate-pyridine-water mixtures proved the most satisfactory and were used for most of the qualitative studies. The ethyl acetate-pyridine-water chromatograms were thoroughly rinsed with anhydrous ethyl ether prior to drying and application of the spray reagent. The 1-butanol-ethanol-water mixture was used for all of the quantitative work. All chromatograms were prepared at room temperature (25°C.) and the total time of irrigation varied from 16 hours to 45 days dependent upon which carbohydrate fractions of the flours were being studied.

The initial spots were placed on the paper by means of a platinum loop which delivered approximately 2 μl. of the concentrate. Continued experience with the flour concentrates made it possible to judge whether more or less than this quantity should be applied to the paper.

The position of the sugars were determined by spraying the dried papers with one of the following reagents: An acidic solution of resorcinol (13), aniline acid phthalate (14), aniline trichloracetic acid (17), or an alkaline solution of 3,5-dinitrosalicylic acid (9, 11). The sugars on the chromatograms were identified by authentic samples of known sugars as controls. This procedure was more dependable than relying on R_f values alone.

Fermentation. Portions of the flour extracts were fermented with bakers' yeast by a previously described method (20), modified by fermenting for a period of 24 hours instead of 3 hours. To confirm the completeness of fermentation, supplementary tests were made in which the yeast nutrient solution employed by Choi *et al.* (8) was included. The results showed that this additional precaution was not necessary under the conditions used in these experiments.

Under the same experimental conditions brewers' yeast, because of its known melibiase activity, was substituted for bakers' yeast, to confirm the identification of raffinose.

The fermented solutions were filtered through Celite, re-deionized with ion-exchange resins, concentrated, and the concentrates were chromatographed as described above.

Quantitative Analysis of Sugars Eluted from the Chromatograms. Portions of the flour concentrates were applied to the filter paper sheets in amounts sufficient to yield enough of each sugar fraction after irrigation and elution to be measurable by the micro copper method of sugar analysis (2). On the same sheet but apart from this strip of sample solution a 2 μl. spot of the same solution was applied, together

with spots of known sugars for controls. On completion of the irrigation period the strip containing the known sugars plus the sample spot was cut off the sheet and sprayed with the proper reagent to mark the positions of the sugars of the sample. With this strip as a guide the rest of the sheet was cut into separate sections of which one contained the fructose and glucose of the sample, one the sucrose fraction, one the maltose fraction, and one contained the sugars from the starting line to maltose. Each of these paper strips was extracted in a soxhlet apparatus with 80% alcohol for at least 4 hours. Experiments indicated that all of the sugars were removed from the filter paper within this period of time. The alcohol was removed from the extracts as previously described, and the sucrose fraction and the fraction from the starting line to maltose were hydrolyzed with 1 *N* hydrochloric acid at room temperature (25°C.) for 24 hours. The reducing power of each of the fractions was measured by a micro copper method (2).

Results and Discussion

Although the four flours studied were from different sources the chromatographic examination of the extracts prepared as described indicated that the same sugars were present in all of them. One very minor exception was observed and this is noted below.

The examination of chromatograms that were irrigated for conventional periods of time showed the presence of fructose, glucose, sucrose, maltose, and an elongated spot at the starting line. The first four sugars are known to be present in wheat flour. The slow moving material was assumed to be the levosine of Tanret (18). The slow movement of this fraction suggested increasing the time of irrigation of the chromatograms beyond that ordinarily used. With long-time irrigation this fraction began to separate on the chromatograms (see Fig. 1). At the end of 45 days at least seven substances, in addition to raffinose, had been separated on the series of chromatograms. Even after this long period of time some material remained at the starting line. This showed that the R_f values of the substances from the starting line to maltose varied by only small increments.

In another experiment invertase solution was added to the sample spot immediately after the mixture had been applied to the chromatographic paper (19). When these chromatograms were completed, fructose, glucose, maltose, melibiose (residue from raffinose), and a spot at the starting line were found. A great increase was noted in the relative amounts of fructose present before and after enzymatic hydrolysis of the mixed sugars.

Table I shows that the sugar values obtained after acid hydrolysis are higher than those obtained after treatment with invertase. This

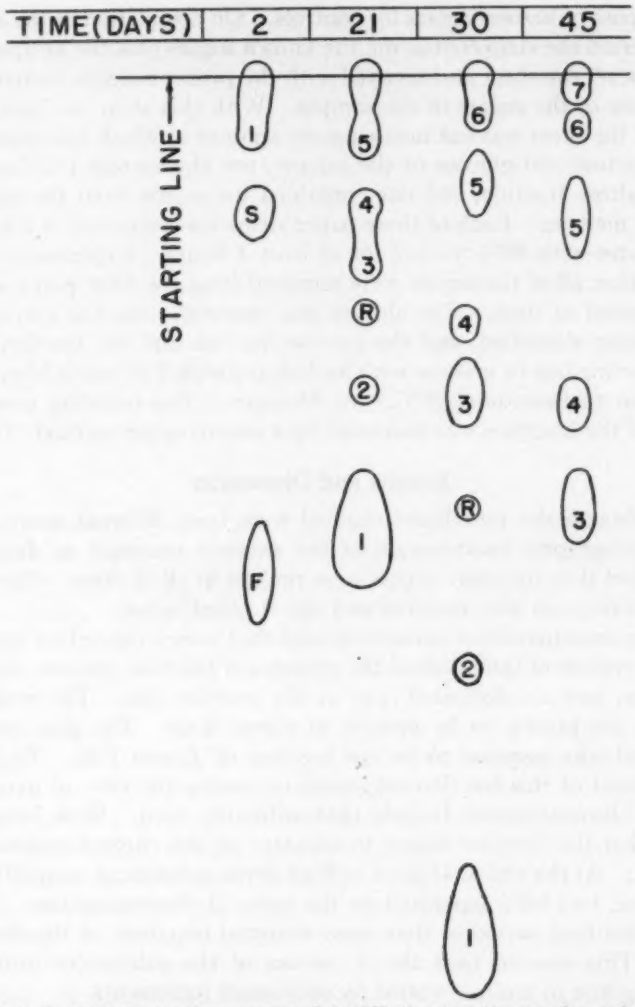

FIG. 1. Facsimile of paper chromatograms of the 80% ethanol-soluble wheat flour carbohydrates sensitive to the resorcinol spray reagent. The solvent mixture used was 1-butanol-ethanol-water (10:1:2). F—fructose, S—sucrose, R—raffinose. The numbers 1 to 7 were assigned to the materials in the order in which they were resolved from the slow-moving spot. On the chromatograms irrigated for 45 days spot 1 had run off of the paper. Spots 2 and R had either run off of the paper or had become too faint, because of diffusion, to be revealed by the spray reagent.

was due, perhaps, to the hydrolysis of the material that remained at the starting line on the chromatogram when the mixed sugars were treated with invertase. Therefore, the mixed sugars were hydrolyzed with acid, chromatographic separations were made, and it was found that no material remained at the starting line. With this exception the chromatograms showed the same sugars by either method of hydrolysis.

Chromatograms of flour extracts which had been fermented with bakers' yeast showed a spot at the starting line, melibiose (residue from raffinose), arabinose, and a small amount of fructose. There was about 0.003% arabinose in three of the flours. This estimation was made by visual comparison of the spots produced by the arabinose from the flour with spots produced by known amounts of this pentose. No arabinose could be detected in the "all-purpose" family type flour. Fermentation experiments were repeated using brewers' yeast. The chromatograms were the same as those prepared from solutions fermented with bakers' yeast except that galactose (residue from raffinose) was found instead of melibiose.

The small amount of fructose present after fermentation suggested the slow hydrolysis of the immobile material since fermentation of solutions of fructose, glucose, and sucrose under similar conditions were known to be complete before 24 hours had elapsed. If the fructose resulted from slow enzyme hydrolysis, then inactivation of the enzyme immediately after the 24-hour fermentation period should give solutions essentially free of fructose. This was found by experiment to be true. Extended treatment of the slowly hydrolyzable immobile material with an enzyme, probably invertase, should bring about complete hydrolysis. This was accomplished by adding solid invertase to a concentrate of the flour extract (containing thymol as a preservative) and storing the solution at room temperature (25°C.) for 17 days. Chromatograms of this solution showed that the immobile spot had completely disappeared; and that the same products formed by 24-hour invertase hydrolysis (which did not affect the bulk of the immobile material) were obtained.

Chromatograms were made on a scale that provided sufficient material for elution and quantitative estimation of certain sugars and sugar groups. The glucose equivalent of the copper reducing power of the fructose-glucose, sucrose, maltose, and the slow moving sugars with R_f values less than maltose were determined. The sucrose and slow moving sugars were acid hydrolyzed before determining the glucose equivalent. The data are given in Table II. The sum of the reducing values of the sugars eluted from the chromatograms represented approximately 95% recovery of the reducing value of the acid-hydrolyzed mixed sugars. The slow moving fraction accounted for about 75% of the reducing power of the mixed sugars.

The experiments show that "levosine" is not a single carbohydrate but a mixture of at least seven substances which have certain common properties. These substances have a ketose structure as indicated by the development of colored spots with the resorcinol spray. None of the other spray reagents used showed additional spots. All of the sub-

TABLE II

QUANTITATIVE ESTIMATION OF SUGARS ELUTED FROM THE PAPER CHROMATOGRAM OF THE "ALL PURPOSE" FAMILY FLOUR

Sugar Fraction	%[1]
Maltose	0.05
Fructose and glucose	0.09
Sucrose	0.21
Origin to maltose[2]	0.82
Total	1.17

[1] As glucose, with a maximum variation of ±2% between replicate analyses.
[2] The slow moving fractions from the starting line of the chromatogram to the maltose fraction, which includes raffinose.

stances contained fructofuranoside linkages, since they were hydrolyzed by yeast invertase. Both the acid and enzymatic hydrolysis of the slow moving sugars gave fructose, glucose, and a trace of melibiose (residue from raffinose). Although various solvent mixtures and spray reagents were used, no other sugars were detected in the hydrolyzates. The total amount of these slow-moving sugars agrees closely with the amount of "levosine" reported in wheat flour by various workers (6). There are conflicting views, however, regarding its fermentability by bakers' yeast either in aqueous solution or in panary fermentation. This confusion apparently results from the difference in behavior of the material that remains at the starting line of the paper chromatogram and the substances that move out slowly and are resolved by extended periods of irrigation. The slow moving substances were easily hydrolyzed by invertase and were completely fermented within 24 hours by bakers' or brewers' yeast, while the immobile fraction was not fermentable and was not readily hydrolyzable by invertase. It is reasonable to assume that these earlier investigators were working with a heterogeneous mixture instead of a pure compound.

Acknowledgments

The authors are indebted to Dr. R. M. McCready for many helpful suggestions regarding chromatographic techniques.

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THE CARBOHYDRATES OF *GRAMINEÆ*. I. THE SUGARS OF THE FLOUR OF WHEAT (*TRITICUM VULGARE*) [1, 2]

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ABSTRACT

Separation of the carbohydrates, extracted from a Southwestern baker's patent wheat flour with aqueous alcohol, by paper partition chromatography followed by analysis using a new colorimetric method has shown that the following sugars are present: glucose (0.01%); fructose (0.02%); sucrose (0.10%); maltose (0.08%) and two slower moving components believed to be melibiose, (0.18%) and raffinose (0.07%). Separation of these sugars could be effected with either 1-butanol-ethanol-water or with 1-butanol-propionic acid-water. Indication of the presence of "levosine" a glucofructosan was forthcoming from the fact that after separation of the mixture of the sugars with 1-butanol-ethanol-water, the slowest moving component was found to provide mainly fructose together with minor amounts of glucose upon hydrolysis with dilute sulfuric acid.

The amount of non-reducing carbohydrates (sometimes called the sucrose value) in the flour, as determined by acid hydrolysis and a modified Hagedron-Jensen ferricyanide procedure, was 1.0%. The discrepancy between this value and the quantity of sucrose found to be present after chromatographic separation is probably due to the presence of a small amount of raffinose and a much larger amount of a non-reducing glucofructosan or levosine.

In connection with an investigation of the fate of various sugars in fermenting doughs and bread made with different flours, formulas and baking procedures, it became necessary to identify and to determine the sugars in wheat flour. The methods heretofore employed in studies of this nature have involved the application of non-specific analytical procedures, such as the measurement of reducing power before and after acid hydrolysis (1, 2, 3, 4, 11, 12) to mixtures of sugars contaminated to a greater or less degree with proteinaceous and lipid-like materials.

Because of their empirical nature, these methods were clearly unsuitable for the present investigations in which precise information was required concerning the identity and amount of each individual sugar. Attention has, therefore, been directed to the application of paper partition chromatographic techniques (6, 9, 13) to the problem of the

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Contribution from the Division of Agricultural Biochemistry, University of Minnesota, Saint Paul, Minnesota. Paper No. 2671 of the Scientific Journal Series, Minnesota Agricultural Experiment Station.

[2] The data in this paper are to be included in a thesis to be submitted to the University of Minnesota by R. B. Koch in partial fulfillment of the requirements for the Ph.D. degree.

separation and determination of the amounts of the various sugars in wheat flour, fermenting doughs and bread. Interest in the sugars of wheat flour was centered particularly on the amount of sucrose present since the glucose and fructose content of freshly-prepared yeast doughs made without added sugars were found to be much too low to account for the value of 1.0% found for the amount of non-reducing sugars ("sucrose") in the original wheat flour by the ferricyanide method of Hagedorn and Jensen (10), as modified by Blish and Sandstedt (3). The work reported herein deals with the qualitative and quantitative analysis of the carbohydrates of a Southwest hard winter wheat flour which are soluble in aqueous alcohol.

Materials and Methods

Extraction of Sugars. Fifty grams of a Southwestern baker's patent wheat flour (protein 11.8%, ash 0.43%, expressed on a 14.0% moisture basis) were suspended in 100 ml. of boiling 70% aqueous ethanol and heated to 80°C. for 7 minutes to inactivate any enzymes. The mixture was cooled, centrifuged, and the supernatant withdrawn. The flour residue was then extracted once with 100 ml. of 30% aqueous ethanol and three times with 100 ml. of distilled water; the extractions were carried out at room temperature and all extracts were combined and the volume measured. The extract (20% with respect to alcohol) was transferred to a 750 ml. Erlenmeyer flask. A small Visking cellophane casing (18/32 in. inflated diameter) containing 10 ml. of 20% aqueous ethanol was placed in the extract which was then rotated (3 r.p.m.) in a plane inclined to the horizontal. Dialysis was allowed to proceed until equilibrium was reached (about 20 hours). The volume of the solution in the Visking casing was measured and then evaporated at 50°C. in vacuo to give a syrupy mixture of sugars. The latter was then dissolved in 1.0 ml. of water.

Chromatographic Analysis of the Sugars. For a preliminary qualitative identification of the sugars, the aqueous solution of the syrupy mixture was subjected to paper partition chromatography using the upper layer of a mixture of 1-butanol-ethanol-water as the irrigating solvent in the usual manner (6, 9). Whatman filter paper No. 1 was used throughout this work. A similar chromatographic analysis was carried out with 1-butanol-propionic acid-water (8). The chromatograms were sparyed either with ammoniacal silver nitrate solution and heated in an oven set at 130°C. or with N,N-dimethyl-p-aminoaniline (0.2 g. N,N-dimethyl-p-aminoaniline hydrochloride and 1.0 g. trichloracetic acid in 50 ml. water) and heated at 130°C. (6). The appearance of spots indicated the presence of sugars which were characterized

by comparison with the spots produced by standard sugars put on the chromatogram at the same time as the unknown mixture.

For the quantitative separation and determination of the sugars, a starting line, eight inches long, was drawn on Whatman No. 1 filter paper and two-inch margins were ruled off from each end of this line, leaving a central portion four inches wide. Within this main portion, 54.4 μl. of the aqueous solution of the syrupy mixture of carbohydrates extracted from the flour were placed along the starting line in a narrow band approximately 0.5 cm. wide. A small amount of this mixture was placed on the starting line in both the marginal strips: in addition, an artificial mixture of the sugars known to be present from the preliminary qualitative tests was also placed on the starting line alongside a small amount of the flour extract. The artificial mixture is particularly useful when the solution being analyzed contains such small amounts of one or more constitutents that it is not feasible to detect them on the marginal strips by spraying unless the paper is overloaded to such an extent with the mixture of sugars at the commencement of the chromatographic experiment that the other spots due to major components overlap. The spots of the mixture of sugars put in the marginal strips must be 1.5 to 2.0 cm. from the central main portion of the chromatogram so as to avoid lateral overlapping.

The chromatogram was then developed for about 60 hours with the upper layer of a mixture of 40 ml. 1-butanol, 10 ml. ethanol and 50 ml. water using the descending technique. The paper was removed, dried in the air at room temperature and the marginal strips cut off and sprayed with a solution of ammoniacal silver nitrate. After five minutes they were heated in an oven at 130°C. until the dark spots indicating the position of the sugars had appeared. The chromatogram was reassembled and, with the aid of lines ruled on the central unsprayed portion of the chromatogram using the spots on the marginal strips as indicators, sections of paper were cut from the central portion so as to include each individual sugar (Fig. 1).

Each of the strips of paper containing a sugar to be determined was immersed in 50 ml. of distilled water for 15 minutes and gentle swirling applied from time to time to aid in the extraction of the sugar. The extracts were then filtered through a plug of glasswool to remove all cellulose fibers and the sugars determined colorimetrically by a phenol-sulfuric acid method recently developed in this laboratory (8). This involves adding 134 μl. of 80% aqueous phenol to 2 ml. of the sugar solution (20 to 40 γ of sugar) followed by the rapid addition of 5 ml. of concentrated sulfuric acid. After thorough mixing, the solution is allowed to stand for about 30 minutes and the optical density measured at 490 mμ in a Junior Coleman spectrophotometer against a blank.

The time of standing is not critical since the color is quite stable. The blank was prepared by extracting a piece of paper of the same size containing no sugar and which was cut from the same chromatogram as that from which the piece of paper containing the sugar had been removed. Each determination was carried out in triplicate. The concentration of sugar was then determined by reference to a standard curve previously established for the particular sugar under examination.

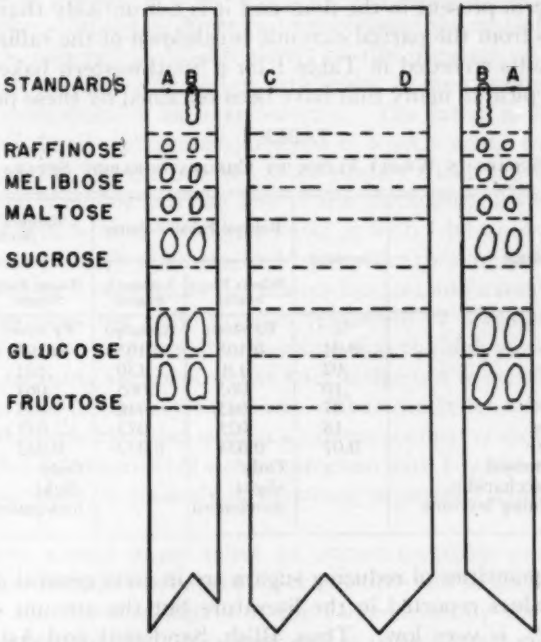

FIG. 1. Diagram showing how a chromatogram is sectioned and developed in determining the individual sugars in a wheat flour extract. A = artificial mixture of pure sugars. B = flour extract, and C to D = aliquot of flour extract.

The polysaccharide (levosine?), which exhibited little movement from the starting line during a 60-hour period of irrigation with the solvent, was extracted from the paper with water. The solution was made 0.01 *N* with respect to sulfuric acid by adding dilute sulfuric acid and heated for 30 minutes in a boiling water bath. The solution was cooled, neutralized by passing through an anion exchange resin "Duolite A4" and evaporated to a small volume. A chromatographic analysis of the sugars present in this solution was made on filter paper using both 1-butanol-ethanol-water and 1-butanol-propionic acid-water as the developing agents.

Results and Discussion

The application of these extraction and chromatographic procedures to wheat flour showed the presence of glucose, fructose, maltose, sucrose, and two slower moving components believed to be melibiose and raffinose inasmuch as their R_f values on 1-butanol-ethanol-water and 1-butanol-propionic acid-water correspond to these two sugars. The raffinose and sucrose are probably, in part at least, derived from traces of wheat germ present in the flour and it is not unlikely that the melibiose arises from the partial enzymic breakdown of the raffinose.

The results recorded in Table I for a Southwestern baker's patent flour are typical of many that have been obtained by these procedures.

TABLE I

THE SUGARS IN WHEAT FLOUR BY CHROMATOGRAPHIC SEPARATION

Sugar	Amount	Butanol-Ethanol-Water		Butanol-Proprionic Acid-Water	
		Wheat Flour Sugars	Authentic Sugars	Wheat Flour Sugars	Authentic Sugars
	%	*Rf values*	*Rf values*	*Rf values*	*Rf values*
Glucose	0.01	0.105	0.105	0.105	0.105
Fructose	.02	.130	.130	.131	.131
Sucrose	.10	.065	.065	.064	.060
Maltose	.07	.045	.046	.045	.045
Melibiose	.18	.025	.023	.021	.020
Raffinoise	0.07	0.034	0.032	0.032	0.030
Undetermined oligosaccharides containing levosine		Only slight movement		Only slight movement	

The total quantities of reducing sugars are in good general agreement with the values reported in the literature but the amount of sucrose found, 0.1%, is very low. Thus, Blish, Sandstedt and Astleford (4) found only about 0.1 to 0.2% reducing sugars in bread flours milled from sound wheat and from 1.0 to 1.47% of "sucrose," based on the determination of reducing sugars before and after inversion. The non-reducing sugar content of this flour, as determined by the ferricyanide procedure of Hagedorn and Jensen (10), as modified by Blish and Sandstedt (3), was 1.1%. Since the acid buffer solution used for the extraction of the sugars from the flour and for the hydrolysis of the sucrose (by heating the extract) has an acidity of pH = 1.4, the high sucrose value obtained by this method may well be due to the hydrolysis of other non-reducing carbohydrates in addition to sucrose.

According to Colin and Belval (5), only a small part of the increase in reducing sugars which results from the acid hydrolysis of a flour extract is due to sucrose; the major portion arising from levosine.

The latter was first isolated from cereals by Tanret (14, 15) and described as a white, amorphous, faintly sweet, easily hydrolyzable, nonfermentable carbohydrate composed chiefly of fructose with a small quantity of glucose. Colin and Belval found that a typical white flour contained 0.17% of hexoses, 0.22% sucrose, and 0.60% levosine.

The results reported herein, obtained by chromatographic techniques therefore support the views of the French workers concerning the low sucrose content of flour (1, 2, 5). The disparity between the values hitherto accepted by American workers and the much lower one found here is believed to be due partly to the presence of the nonreducing trisaccharide raffinose but principally to the presence of nonreducing polysaccharides such as levosine. The latter is soluble in aqueous alcohol and undergoes hydrolysis in weakly acidic conditions. The component of the mixture of carbohydrates which moved only a short distance from the starting line of the chromatogram when irrigated either with 1-butanol-ethanol-water or with 1-butanol-propionic acid-water was partially characterized by the fact that upon hydrolysis with 0.01 *N* sulfuric acid it afforded mainly fructose and a small amount of glucose as does the polyfructosan (graminin or levosine) of rye flour (7). It seems very probable therefore that the high value of 1.0% for the non-reducing sugars of wheat flour is due not to sucrose but to one or more acid-labile polyfructosans. That the latter slow-moving fraction of the carbohydrates is not a single component is shown by the fact that upon development of a chromatogram with 1-butanol-ethanol-water for four days, at least six incompletely separated spots could be discerned.

The above results based solely on chromatographic analysis are tentative ones and conclusive proof of the identity of the various sugars must await the characterization of the components by the isolation of crystalline compounds. This work is now in progress.

Acknowledgment

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BOOK REVIEWS

Official Methods of Analysis of the Association of Official Agricultural Chemists. Seventh Edition. XV + 910 pages, 73 illustrations and 31 reference tables. Published by the A.O.A.C., P. O. Box 540, Benjamin Franklin Station, Washington 4, D. C. 1950. Price per copy domestic $10.00, foreign $10.50.

The seventh edition of this well-known and authoritative compilation of methods for the analysis of agricultural, food, and drug products contains procedures which have been sponsored by the Association up to and including those adopted at its 1949 meeting.

Earlier editions of this work have borne the name *Official and Tentative Methods of Analysis* and the title of the present edition reflects the recent action of the Association in deleting the "tentative" classification. As explained in the preface to the current edition, "methods are now designated 'first action' upon first adoption and 'official' upon subsequent final adoption. Thus is continued the long-established custom of providing a year's notice to all who may be interested (by publication of a method upon first adoption) that final adoption is pending, thereby permitting opportunity for such further critical study as may be desirable before final approval of the official status is granted."

In this latest edition, the chapters on naval stores, leathers and tanning materials have been deleted as well as the section of the appendix of the sixth edition which dealt with the definitions of terms and interpretation of the results of analyses of fertilizers and liming materials. A chapter entitled "Economic Poisons" has been substituted for "Insecticides and Fungicides" of previous editions and includes methods for the analysis of insecticides, fungicides, herbicides, rodenticides and disinfectants. The chapters on cosmetics; coloring matters; metals, other elements and residues in foods; preservatives; vitamins; and extraneous materials in foods and drugs have been considerably enlarged with a slight decrease in the total number of pages. Although the process of collaborative testing of methods included in this manual is very time-consuming, several of the newer analytical techniques, such as spectrophotometry and chromatography as well as new procedures for the determination of certain substances, such as 2,4-dichlorophenoxyacetic acid and pteroylglutamic acid are included.

This book not only includes methods which are required in regulatory work but also those which are useful in chemical research. The book is remarkably free from typographical errors and is an essential reference work for the food chemist.

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Macaroni Products. By Charles Hummel. 223 pp. Food Trade Press, Ltd., London, England, 1950. Price $6.20 U.S.A. and Canada; £2 Sterling England.

This book gives a rather complete description of the manufacture, processing, and packing of macaroni products. The term macaroni products is used in an all inclusive sense and refers to paste products such as macaroni, spaghetti, vermicelli, noodles, etc. There has been a demand for a technical book covering the manufacture of macaroni products and the specialized machinery used in this industry. We believe this book is the first complete publication ever written in the English language on this subject. Its recent release allows for inclusion, a description of machinery and manufacturing techniques for the continuous producing macaroni, spaghetti and noodle machines which have made a very rapid entrance into this field in the last ten years.

The subject matter of the 13 chapters included in this book is as follows: (1) the importance of macaroni products and historical notes; (2) manufacture of macaroni products; (3) ingredients used in the manufacture of macaroni products,

semolina, water, eggs, other products; (4) batch manufacturing process; (5) continuous manufacturing process; (6) some typical continuous extrusion presses; (7) drying macaroni products; (8) the quality of macaroni products and how it can be tested; (9) storing and packing macaroni products; (10) the ideal macaroni plant; (11) notes on the theory of drying.

The author has relied heavily on previously published literature and contacts with the machine manufacturers for the material presented in this book. The subject matter is quite complete and has been presented in such a way that it will be of interest to both those conversant with the manufacture of macaroni products, as well as those who are not. Each function carried out in the processing of macaroni products from raw material through dough processing and drying of the macaroni products, as well as the packaging of such products, is covered in detail. All of these functions emphasize the importance of control for the production of quality products.

This book carries many photographs of dough processing and drying machines. The author has been misinformed as to the present definition of semolina and farina in the U.S.A. The present standards for these products allow for a maximum of 15% moisture and 3% flour, whereas the author presented the old maximum standard of 13.5% moisture and 1% flour.

This book will be of interest to those who would like to gain a general knowledge of the macaroni products industry, and will be of technical value to present manufacturers of macaroni products.

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Colloid Dispersions. By Earl K. Fischer, National Bureau of Standards. John Wiley and Sons, Inc., New York, N. Y. 1950. 387 pp. 15 × 23 cm. Price, $7.50.

This volume sets forth, in an instructive and well-coordinated manner, the theoretical aspects and manufacturing details that are involved in the industrially important processes currently employed in the preparation of a wide variety of products containing pigments, carbon blacks, clays and other solids in a fine state of subdivision in liquid media. The desirable and undesirable properties of such products are interpreted in terms of surface and colloid chemical theory and related to conditions and procedures of manufacture. References to scientific periodicals, including trade journals, are entensive and a wealth of patent literature is integrated into the discussion.

Following a short introduction, four chapters present theoretical concepts that are of particular importance to a discussion of the properties of matter in the state of colloidal dispersion. Particle size, the solid-liquid interface and wetting, the state of the dispersed solid and rheological properties of dispersions are the subjects treated. For each, methods for obtaining and interpreting data in relation to desired properties in the dispersion are discussed and evaluated. Some 220 pages of the volume are devoted to these chapters. Included are discussions of many ramifications and industrial examples illustrative of the subject matter treated. A chapter on surface active agents reviews the nature of these diversified materials and offers some guiding principles to be followed in promoting, by their use, certain desired properties in the various types of dispersions. Two short chapters on the process of comminution and on mixing are followed by chapters in which the operations of roll mills, ball and pebble mills and disc, cone and colloid mills are described in some detail. A final chapter discusses the industrially important process of dispersion by phase transfer—the flushing process.

The author's purpose of assembling and integrating the essential information obtained from the many different fields of research and technology which are concerned with the preparation, improvement and utilization of systems of colloidally dispersed solids in liquid media is quite adequately accomplished. The volume will serve as a valuable data source and process guide to industrial chemists who are engaged in the manufacture of paints, ceramics, textile colors and printers ink, to mention only a few products in which the colloidal dispersion of solids in liquids is of primary importance. Chemists who are interested in any phase of colloid science will

find this book profitable reading in its function of correlating theory and practice in that field of study.

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The Practice and Science of Bread-Making. By D. W. Kent-Jones and John Price, The Northern Publishing Co., Ltd., Liverpool 1, England. 1951. 278 pp. Price, 25 shillings.

The Practice and Science of Bread-Making is intended for the practical baker and for students of baking technology rather than for the scientifically trained laboratory worker. The chapters on history of bread-making, wheat and flour, dough ingredients other than flour, dough testing apparatus, principles of fermentation, bread-making processes, bread faults, nutritive value of bread, and analytical work are as valuable for readers in America as for those in England. Fundamental principles such as these do not vary from one country to another. Perhaps three of the fourteen chapters would not be of practical value to Americans (brown and fancy bread, fermented confectionery, and bakehouse management), yet these chapters are certainly of general interest.

The book is well printed on good quality paper. It contains forty-three excellent illustrations and a number of tables.

This book would be of value to all bakeshop foremen and production men in the industry and as an adjunct reference book for students of milling and baking. It also should be in the library of all baking and milling chemists. The problem of interpretation of technical findings to management is faced by all technical workers. This book offers many valuable interpretations of scientific findings in readily understood terminology. There are very few manuals and textbooks on baking science and this present book is indeed helpful in augmenting the available material.

GASTON DALBY
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